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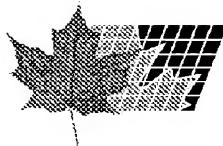
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(71)	SOUTH ALABAMA MEDICAL SCIENCE FOUNDATION, University of South Alabama	

(54) CANAL CALCIQUE DE TYPE T
(54) T-TYPE CALCIUM CHANNEL

(57)

The present invention is directed to isolated nucleic acid molecules encoding pancreatic T-type calcium channels and vectors and host cells comprising such. The invention is further directed to methods and compositions which modulate the expression of pancreatic T-type calcium channels, including antisense. An isolated pancreatic T-type calcium channel protein is provided, as well as antibodies directed to such protein. Pharmaceutical compositions and methods of treatment involving pancreatic T-type calcium channels are also provided.



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(71) Applicant:	SOUTH ALABAMA MEDICAL SCIENCE FOUNDATION [US/US]; P.O. Box U-1060, Mobile, AL 36688 (US).		
(72) Inventor:	LI, Ming, 7231 Bay Road, Mobile, AL 36605 (US).		
(74) Agents:	BRAMAN, Susan, J. et al.; Braman & Rogalskyj, LLP, P.O. Box 352, Canandaigua, NY 14424-0352 (US).		
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Published*With international search report.**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.***(54) Title:** T-TYPE CALCIUM CHANNEL**(57) Abstract**

The present invention is directed to isolated nucleic acid molecules encoding pancreatic T-type calcium channels and vectors and host cells comprising such. The invention is further directed to methods and compositions which modulate the expression of pancreatic T-type calcium channels, including antisense. An isolated pancreatic T-type calcium channel protein is provided, as well as antibodies directed to such protein. Pharmaceutical compositions and methods of treatment involving pancreatic T-type calcium channels are also provided.

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T-TYPE CALCIUM CHANNEL

This application claims priority of U.S. Provisional Patent Application No. 60/098,004, filed August 26, 1998,
5 and of U.S. Provisional Patent Application No.
60/117,399, filed January 27, 1999.

The subject matter of this application was made with support from the United States Government under National
10 Institutes of Health Grant No. 5-20174. The U.S. Government may have certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates generally to calcium
15 channel proteins, and more particularly to pancreatic T-type calcium channel proteins and uses thereof.

BACKGROUND OF THE INVENTION

Throughout this application various publications are
20 referenced, many in parenthesis. Full citations for each of these publications are provided at the end of the Detailed Description. The disclosures of each of these publications in their entireties are hereby incorporated by reference in this application.

25 Insulin secretion from pancreatic β -cells is the primary physiological mechanism of blood glucose regulation. A rise in blood glucose concentration stimulates release of insulin from the pancreas, which in turn promotes glucose uptake in peripheral tissues and
30 consequently lowers blood glucose levels, reestablishing euglycemia. Non-insulin dependent diabetes mellitus (NIDDM) (type II diabetes) is associated with an impairment in glucose-induced insulin secretion in pancreatic β -cells (Vague and Moulin, 1982).

35 Voltage-gated Ca^{2+} channels mediate a rapidly activated inward movement of Ca^{2+} ions that underlies the

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stimulation of insulin secretion in β -cells (Boyd III 1991). In different tissues, four types of Ca^{2+} channels have been described (L(P/Q), T, N, and E channels). The purified L-type Ca^{2+} channel consists of five subunits: α_1 , 5 α_2 , β , γ , δ (Catterall 1991). The primary structure of the α_1 subunit is organized in four homologous domains containing six transmembrane segments (Catterall 1988).

Rat and human pancreatic β -cells are equipped with L-type and T-type Ca^{2+} channels (Hiriart and Matteson, 10 1988; Davalli et al., 1996). L-type Ca^{2+} channels, activated at high voltages and having large unitary conductance and dihydropyridine-sensitivity, are considered the major pipeline for Ca^{2+} influx into the β -cell (Keahey et al., 1989). In contrast, T-type calcium 15 channels activate at low voltages and have small unitary conductance and dihydropyridine-insensitivity.

The physiological function of T-type Ca^{2+} channels in β -cell insulin-secretion has been demonstrated (Bhattacharjee et al., 1997). These channels facilitate 20 exocytosis by enhancing electrical activity in these cells. L-type and T-type Ca^{2+} channels, under normal conditions, work in concert promoting the rise in $[\text{Ca}^{2+}]_i$ during glucose-stimulated insulin secretion. In β -cells, over-expressed T-type Ca^{2+} channels may be, at least in 25 part, responsible for the hyper-responsiveness of insulin secretion to non-glucose depolarizing stimuli in GK rat and in rat with NIDDM induced by neonatal injection of streptozotocin (Kato et al., 1994; Kato et al., 1996). However, over-expressed T-type calcium channels over time 30 will ultimately lead to an elevation of basal Ca^{2+} through it's window current properties. Therefore, there is a dual effect of T-type Ca^{2+} channels in β -cells depending upon channel number and membrane potential.

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Two isoforms of L-type Ca^{2+} channel $\alpha 1$ subunits have been identified in β -cells (Seino et al., 1992; Yaney et al., 1992). The rat neuronal T-type calcium channel has recently been cloned (Perez-Reyes et al., 1998). Other 5 subunits of T-type Ca^{2+} channel have yet to be identified.—

Given the evidence that T-type calcium channels are associated with type II diabetes, a need exists to further characterize T-type calcium channels.

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SUMMARY OF THE INVENTION

To this end, the subject invention provides an isolated nucleic acid molecule encoding a pancreatic T-type calcium channel. The invention also provides an antisense nucleic acid molecule complementary to at least 15 a portion of the mRNA encoding the pancreatic T-type calcium channel.

The isolated nucleic acid molecules of the invention can be inserted into suitable expression vectors and/or host cells. Expression of the nucleic acid molecules 20 encoding the pancreatic T-type calcium channel results in production of pancreatic T-type calcium channel in a host cell. Expression of the antisense nucleic acid molecules in a host cell results in decreased expression of the pancreatic T-type calcium channel.

25 The invention further provides a ribozyme having a recognition sequence complementary to a portion of mRNA encoding a pancreatic T-type calcium channel. The ribozyme can be introduced into a cell to also achieve decreased expression of pancreatic T-type calcium channel 30 in the cell.

The invention further provides a method of screening a substance for the ability of the substance to modify T-type calcium channel function, and a method of obtaining DNA encoding a pancreatic T-type calcium channel.

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Further provided is an isolated nucleic acid molecule encoding a pancreatic T-type calcium channel, wherein the nucleic acid molecule encodes a first amino acid sequence having at least 90% amino acid identity to 5 a second amino acid sequence. The second amino acid sequence is as shown in SEQ ID NO:2.

The invention further provides a DNA oligomer capable of hybridizing to a nucleic acid molecule encoding a pancreatic T-type calcium channel. The DNA 10 oligomer can be used in a method of detecting presence of a pancreatic T-type calcium channel in a sample, which method is also provided by the subject invention.

The invention also provides an isolated pancreatic T-type calcium channel protein, and antibodies or 15 antibody fragments specific for the pancreatic T-type calcium channel protein. The antibodies and antibody fragments can be used to detect the presence of the pancreatic T-type calcium channel protein in samples.

Further provided is an isolated pancreatic T-type calcium 20 channel protein encoded by a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence as shown in SEQ ID NO:2.

The subject invention further provides a method of 25 modifying insulin secretion by pancreatic beta cells, the method comprising modifying levels of functional T type calcium channels in the pancreatic beta cells. The invention further provides a method of treating type II diabetes in a subject, the method comprising 30 administering to the subject an amount of a compound effective to modify levels of functional T type calcium channel in the pancreatic beta cells of the subject.

The invention also provides a method of modifying basal calcium levels in cells, a method of modifying the

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action potential of L type calcium channels in cells, a method of modifying pancreatic beta cell death, a method of modifying pancreatic beta cell proliferation, and a method of modifying calcium influx through L type calcium 5 channels in cells, each of the methods comprising — modifying levels of functional T type calcium channels in the cells.

BRIEF DESCRIPTION OF THE DRAWINGS

10 These and other features and advantages of this invention will be evident from the following detailed description of preferred embodiments when read in conjunction with the accompanying drawings in which:

Fig. 1A illustrates a comparison of the nucleotide 15 sequence of α_1 G-INS (1) and α_1 G (2) at the 5'-end regions (aa1-67 of α_1 G). The four insertions are indicated with arrow heads. The capital ATG represents the start codon for each cDNA;

Fig. 1B is a schematic illustration representing 20 partial rat genomic nucleotide composition between Domain III and IV. Genomic DNA contained an exon specific to α_1 G (shaded circle) and an exon specific to the α_1 subunit of T-type Ca^{2+} deduced from INS-1 (shaded rectangle) between 4845 and 5256 of the cDNA sequence. Other exons (open 25 rectangles) are identical between the two cDNAs. The bold letters indicate the nucleotides coding Gly-1667;

Figs. 2A-2D illustrate expression of α_1 G-INS in Xenopus oocytes. Fig. 2A illustrates 40 mM Ca^{2+} currents elicited by depolarizing pulses from - 60 to 40 mV. Fig. 30 2B illustrates time constants of activation and inactivation measured at test potentials between -30 and 30 mV. The time constants of activation were obtained by fitting the increasing portion (activation) of currents with the Hodgkin-Huxley equation where the m value was

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designated as four ($n = 6$). The time constants of inactivation were obtained by single exponential fitting ($n = 6$). Fig. 2C illustrates voltage-dependent conductance ($n = 7$) and Fig. 2D illustrates steady-state 5 inactivation ($n = 3$) of expressed currents in oocytes. — The holding potential for Figs. 2C and 2D was -80 mV. The currents in Fig. 2D were measured at -10 mV after varying 1000 ms pre-pulse potentials. Peak currents were normalized to the maximum current and then averaged 10 (error bars represent SE);

Figs. 3A and 3B illustrate accumulative dose response relationships of the inhibitory effects of mibepradil on T- and L-type Ca^{2+} currents. Currents were measured with the whole-cell patch clamp configuration. 15 Data from four experiments were normalized individually and than plotted as mean \pm standard error. Fig. 3A illustrates curve which was generated by fitting the data using one-to-one binding curve according to the equation $1/(1 + [\text{mibepradil}]/K_d)$. Fig. 3B is a dose response of 20 L-type Ca^{2+} current obtained when perfusion of solutions containing different concentrations of mibepradil;

Fig. 4 illustrates reversibility of the inhibition of T and L-type currents by NiCl_2 and mibepradil, respectively. Open and solid circles represent the T-type 25 Ca^{2+} current recorded before and after NiCl_2 (2 μl of 30 μM) and mibepradil (2 μl of 10 μM) were administrated, respectively. The open squares represent the L-type Ca^{2+} current recorded before and after mibepradil (2 μl of 10 μM) was administrated with perforated patch clamp 30 configuration. The T-type Ca^{2+} current was measured at -30 mV with a holding potential of -80 mV with whole cell configuration. Arrow indicates the time when the drugs were delivered. $n = 3$ for each group experiments;

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Figs. 5A and 5B illustrate the long-term effect of mibepradil (10 nM) on L- and T- Ca^{2+} currents in the perforated-patch configuration. In Fig. 5A, solid and open circles represent the L-type Ca^{2+} current recorded in the cells with and without administration of mibepradil, respectively. Solid triangles represent T-type Ca^{2+} currents recorded in the cells after administrating mibepradil. Mibepradil were delivered at time zero. n = 4 for each group experiments. In Fig. 5B, cells were cultured in medium with or without co-incubating 10 nM mibepradil for 2 hours. The current densities were recorded with perforated patch clamp configuration. n = 14 for each group experiments;

Fig. 6A illustrates accumulation of dm-mibepradil in the cells measured with mass spectrometry. The cells were first incubated with mibepradil (20 μM) for the duration indicated on the figure (n = 3). The inset (Fig. 6B) shows the primary data of mass spectrometry indicating peaks at 496 and 424, which correspond to mibepradil and dm-mibepradil, respectively;

Fig. 7A illustrates the effect of mibepradil and dm-mibepradil on L-type Ca^{2+} currents from inside cells. n = 8, *, p < 0.01 to the control;

Fig. 7B illustrates the effect of mibepradil or dm-mibepradil on T-type Ca^{2+} current from inside cells n = 4. All data were collected at 5 min after formation of whole cell patch. The pipette solution contained 1 μM of drug;

Fig. 8 illustrates basal $[\text{Ca}^{2+}]_i$ measured in an INS-1 cell. T-type calcium channel antagonist mibepradil (1 μM) reduced basal $[\text{Ca}^{2+}]_i$ in a single cell in the bath solution without glucose. The $[\text{Ca}^{2+}]_i$ was measured with the emission ratio of Fura-2 AM (F380/F340) then

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calibrated with the standard solution purchased from Molecular Probes Inc. (OR);

Fig. 9A illustrates that intracellular perfusion of a solution containing 272 nM free calcium concentration 5 inhibits the L-type calcium current. Currents were elicited by a step voltage to +10 mV, with holding potential of -80 mV;

Fig. 9B illustrates the effect of perfusing in high calcium concentration on the IV calcium current 10 relationship. Closed circles represent the cell before perfusion, and open circles represent perfusion of 272 nM free calcium;

Fig. 9C illustrates the effect of intracellular perfusion of different calcium concentrations on L-type 15 calcium current over time. Squares represent perfusion from high calcium to low calcium (intracellular solution contained 632 nM then perfused by a solution with 10 mM EGTA), triangles represent perfusion from low calcium to 272 nM calcium, and circles represent low calcium to 632 20 nM calcium;

Fig. 9D illustrates the effect of high calcium on the T-type calcium channel current. Tail currents were elicited by a voltage step to -30 mV for 10 ms;

Fig. 10 illustrates that reestablishment of basal 25 calcium causes stereotyped calcium influx. A cell was twice perfused with 50 mM KCl with an intervening perfusion of the original bath solution to restore membrane potential;

Fig. 11 illustrates that elevated basal Ca^{2+} causes a 30 defect in the Ca^{2+} transient. A cell was twice perfused with 50 mM KCl with an intervening perfusion of the original bath solution to restore membrane potential. The second perfusion occurred prior to reestablishment of the original basal $[\text{Ca}^{2+}]_i$ of about 60 nM;

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Fig. 12 illustrates a model for glucose-stimulated insulin release;

Fig. 13 illustrates that mibefradil ($1 \mu\text{M}$) blocks T- and L-type Ca^{2+} current in INS-1 cells. The relative current of T type Ca channel is obtained by measuring their slow deactivated tail current ($n = 8$);

Fig. 14 illustrates that mibefradil and NiCl_2 reversibly block T type Ca^{2+} current in INS-1 cells.

Drugs were administered into the recording chamber at 180 seconds from the beginning of recording. $N = 3$;

Fig. 15 illustrates the activation and inactivation curves for INS-1 cells, revealing a "window current";

Fig. 16 illustrates the effect of NiCl_2 , mibefradil, and nifedipine on basal insulin secretion in NIT-1 cells. The glucose concentration is 3 mM in the experiments;

Fig. 17 illustrates that the T type calcium channel antagonist NiCl_2 ($30 \mu\text{M}$) reduced the frequency of transient spontaneous elevation of $[\text{Ca}^{2+}]_i$, in a single cell in the bath solution without glucose;

Fig. 18 illustrates the effect of 30 mM NiCl_2 on the $[\text{Ca}^{2+}]_i$ under non-stimulus conditions. Data was collected from the cells with "high" initial basal $[\text{Ca}^{2+}]_i$ (about 100 nM). $n = 13$;

Figs. 19A and 19B illustrate that hyperpolarization induced an increase in number of action potentials and a decrease in onset latencies. $N = 40$;

Figs. 20A and 20B illustrate the dose-dependent effect of NiCl_2 on insulin secretion. Cells were placed in a medium containing 11.1 mM glucose and a decrease in onset latencies. $N = 40$;

Fig. 21 illustrates "run-up" in whole cell recording;

Fig. 22 illustrates KCl induced Ca^{2+} influx in the INS-1 cells treated with streptozotocin. $n = 13$;

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Fig. 23A-23F illustrate the results of cytokine treatment. LVA Ca^{2+} currents were induced by cytokine treatment (IL-1 β , 25 U/ml; IFN γ , 300 U/ml) for 6 h in primary cultured mouse islet cells, but not in α -TC1 cells. An LVA current was elicited by a -40 mV test pulse in an islet cell (Fig. 23A), but the same current was not detected in α -TC1 cells (Fig. 23C). The Ca^{2+} current density-voltage relationships obtained from islet cells (Fig. 23B) and α -TC1 cells (Fig. 23D) with and without cytokine treatment are shown. The open circles represent the current densities of untreated cells ($n = 10$ for islet cells; $n = 20$ for α -TC1 cells), and the filled circles represent the current densities of cells treated by cytokines ($n = 21$ for islet cells; $n = 21$ for α -TC1 cells). The recordings were elicited by voltages ranging from -50 to +20 mV for 100 msec. All experiments were performed at -80 mV. Fig. 23E shows steady state inactivation of LVA tail currents elicited by a 10-msec depolarizing (-10 mV) pulse followed by a 50-msec hyperpolarizing pulse (-100 mV), with a holding potential of -80 mV. Fig. 23F shows that NiCl_2 (10 μM) blocked the cytokine induced LVA Ca^{2+} current elicited at a -30 mV step pulse in an islet cell;

Figs. 24A and 24B illustrate the effects of cytokines on $[\text{Ca}^{2+}]_i$ in mouse islet cells and α -TC1 cells. In Fig. 24A, basal $[\text{Ca}^{2+}]_i$ of primary cultured mouse islet cells was approximately 3-fold higher after cytokine treatment. NiCl_2 (10 μM), but not nifedipine (10 μM), prevented the increase in $[\text{Ca}^{2+}]_i$. In Fig. 24B, basal $[\text{Ca}^{2+}]_i$ in α -TC1 cells was unaffected by cytokine treatment. Cytokine treatment consisted of IL-1 β (25 U/ml) and IFN γ (300 U/ml) for 6 h; and

Figs. 25A and 25B illustrate the effects of NiCl_2 on cytokine-induced β -TC3 cell death. NiCl_2 (20 μM)

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significantly reduced cell death induced by cytokines in both a time (Fig. 25A) and dose-dependent (Fig. 25B) manner (n = 3). Cytokine treatment consisted of IL-1 β (25 U/ml), IFN γ (100 U/ml), and TNF α (100 U/ml) in Fig. 5 25A and of IL-1 β (25 U/ml), TNF α (100 U/ml), and various concentrations of IFN γ as indicated in Fig. 25A. The first dose, 0, represents zero concentration for all three cytokines. The concentration of nifedipine was 10 μ M in both Fig. 25A and Fig. 25B.

10

DETAILED DESCRIPTION OF THE INVENTION

The term "nucleic acid", as used herein, refers to either DNA or RNA. "Nucleic acid sequence" or "polynucleotide sequence" refers to a single- or 15 double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. It includes both self-replicating plasmids, infectious polymers of DNA or RNA, and nonfunctional DNA or RNA.

"Isolated" nucleic acid refers to nucleic acid which 20 has been separated from an organism in a substantially purified form (i.e. substantially free of other substances originating from that organism), and to synthetic nucleic acid.

By a nucleic acid sequence "homologous to" or 25 "complementary to", it is meant a nucleic acid that selectively hybridizes, duplexes or binds to DNA sequences encoding the protein (channel) or portions thereof when the DNA sequences encoding the protein are present in a human genomic or cDNA library. A DNA 30 sequence which is similar or complementary to a target sequence can include sequences which are shorter or longer than the target sequence so long as they meet the functional test set forth.

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Typically, the hybridization is done in a Southern blot protocol using a 0.2X SSC, 0.1% SDS, 65°C wash. The term "SSC" refers to a citrate-saline solution of 0.15M sodium chloride and 20 mM sodium citrate. Solutions are often expressed as multiples or fractions of this concentration. For example, 6X SSC refers to a solution having a sodium chloride and sodium citrate concentration of 6 times this amount or 0.9 M sodium chloride and 120 mM sodium citrate. 0.2X SSC refers to a solution 0.2 times the SSC concentration or 0.03M sodium chloride and 4 mM sodium citrate.

The phrase "nucleic acid molecule encoding" refers to a nucleic acid molecule which directs the expression of a specific protein or peptide. The nucleic acid sequences include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein or peptide. The nucleic acid molecule includes both the full length nucleic acid sequences as well as non-full length sequences derived from the full length protein. It being further understood that the sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell.

The term "located upstream" as used herein refers to linkage of a promoter upstream from a nucleic acid (DNA) sequence such that the promoter mediates transcription of the nucleic acid (DNA) sequence.

The term "vector", refers to viral expression systems, autonomous self-replicating circular DNA (plasmids), and includes both expression and nonexpression plasmids. Where a recombinant microorganism or cell is described as hosting an "expression vector," this includes both extrachromosomal

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circular DNA and DNA that has been incorporated into the host chromosome(s). Where a vector is being maintained by a host cell, the vector may either be stably replicated by the cells during mitosis as an autonomous structure, or the vector may be incorporated within the host's genome.

The term "plasmid" refers to an autonomous circular DNA molecule capable of replication in a cell, and includes both the expression and nonexpression types.

Where a recombinant microorganism or cell is described as hosting an "expression plasmid", this includes latent viral DNA integrated into the host chromosome(s). Where a plasmid is being maintained by a host cell, the plasmid is either being stably replicated by the cell during mitosis as an autonomous structure, or the plasmid is incorporated within the host's genome.

The phrase "heterologous protein" or "recombinantly produced heterologous protein" refers to a peptide or protein of interest produced using cells that do not have an endogenous copy of DNA able to express the peptide or protein of interest. The cells produce the peptide or protein because they have been genetically altered by the introduction of the appropriate nucleic acid sequences. The recombinant peptide or protein will not be found in association with peptides or proteins and other subcellular components normally associated with the cells producing the peptide or protein.

The following terms are used to describe the sequence relationships between two or more nucleic acid molecules or polynucleotides, or between two or more amino acid sequences of peptides or proteins: "reference sequence", "comparison window", "sequence identity", "sequence homology", "percentage of sequence identity", "percentage of sequence homology", "substantial

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identity", and "substantial homology". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a 5 full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence.

Optimal alignment of sequences for aligning a comparison window may be conducted, for example, by the local homology algorithm of Smith and Waterman (1981), by 10 the homology alignment algorithm of Needleman and Wunsch (1970), by the search for similarity method of Pearson and Lipman (1988), or by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics 15 Computer Group, 575 Science Dr., Madison, Wis.).

As applied to nucleic acid molecules or polynucleotides, the terms "substantial identity" or "substantial sequence identity" mean that two nucleic acid sequences, when optimally aligned (see above), share 20 at least 90 percent sequence identity, preferably at least 95 percent sequence identity, more preferably at least 96, 97, 98 or 99 percent sequence identity.

"Percentage nucleotide (or nucleic acid) identity" or "percentage nucleotide (or nucleic acid) sequence 25 identity" refers to a comparison of the nucleotides of two nucleic acid molecules which, when optimally aligned, have approximately the designated percentage of the same nucleotides. For example, "95% nucleotide identity" refers to a comparison of the nucleotides of two nucleic 30 acid molecules which when optimally aligned have 95% nucleotide identity. Preferably, nucleotide positions which are not identical differ by redundant nucleotide substitutions (the nucleotide substitution does not change the amino acid encoded by the particular codon).

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As further applied to nucleic acid molecules or polynucleotides, the terms "substantial homology" or "substantial sequence homology" mean that two nucleic acid sequences, when optimally aligned (see above), share 5 at least 90 percent sequence homology, preferably at least 95 percent sequence homology, more preferably at least 96, 97, 98 or 99 percent sequence homology.

"Percentage nucleotide (or nucleic acid) homology" or "percentage nucleotide (or nucleic acid) sequence 10 homology" refers to a comparison of the nucleotides of two nucleic acid molecules which, when optimally aligned, have approximately the designated percentage of the same nucleotides or nucleotides which are not identical but differ by redundant nucleotide substitutions (the 15 nucleotide substitution does not change the amino acid encoded by the particular codon). For example, "95% nucleotide homology" refers to a comparison of the nucleotides of two nucleic acid molecules which when optimally aligned have 95% nucleotide homology.

20 As applied to polypeptides, the terms "substantial identity" or "substantial sequence identity" mean that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap, share at least 90 percent sequence identity, preferably at least 25 95 percent sequence identity, more preferably at least 96, 97, 98 or 99 percent sequence identity.

"Percentage amino acid identity" or "percentage amino acid sequence identity" refers to a comparison of the amino acids of two polypeptides which, when optimally 30 aligned, have approximately the designated percentage of the same amino acids. For example, "95% amino acid identity" refers to a comparison of the amino acids of two polypeptides which when optimally aligned have 95% amino acid identity. Preferably, residue positions which

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are not identical differ by conservative amino acid substitutions. For example, the substitution of amino acids having similar chemical properties such as charge or polarity are not likely to affect the properties of a 5 protein. Examples include glutamine for asparagine or — glutamic acid for aspartic acid.

As further applied to polypeptides, the terms "substantial homology" or "substantial sequence homology" mean that two peptide sequences, when optimally aligned, 10 such as by the programs GAP or BESTFIT using default gap, share at least 90 percent sequence homology, preferably at least 95 percent sequence homology, more preferably at least 96, 97, 98 or 99 percent sequence homology.

"Percentage amino acid homology" or "percentage 15 amino acid sequence homology" refers to a comparison of the amino acids of two polypeptides which, when optimally aligned, have approximately the designated percentage of the same amino acids or conservatively substituted amino acids. For example, "95% amino acid homology" refers to 20 a comparison of the amino acids of two polypeptides which when optimally aligned have 95% amino acid homology. As used herein, homology refers to identical amino acids or residue positions which are not identical but differ only by conservative amino acid substitutions. For example, 25 the substitution of amino acids having similar chemical properties such as charge or polarity are not likely to affect the properties of a protein. Examples include glutamine for asparagine or glutamic acid for aspartic acid.

30 The phrase "substantially purified" or "isolated" when referring to a protein (or peptide), means a chemical composition which is essentially free of other cellular components. It is preferably in a homogeneous state although it can be in either a dry or aqueous

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solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein (or peptide) which is 5 the predominant species present in a preparation is substantially purified. Generally, a substantially purified or isolated protein (or peptide) will comprise more than 80% of all macromolecular species present in the preparation. Preferably, the protein (or peptide) is 10 purified to represent greater than 90% of all macromolecular species present. More preferably the protein (or peptide) is purified to greater than 95%, and most preferably the protein (or peptide) is purified to essential homogeneity, wherein other macromolecular 15 species are not detected by conventional techniques. A "substantially purified" or "isolated" protein (or peptide) can be separated from an organism, synthetically or chemically produced, or recombinantly produced.

"Biological sample" or "sample" as used herein 20 refers to any sample obtained from a living organism or from an organism that has died. Examples of biological samples include body fluids and tissue specimens.

High stringent hybridization conditions are selected at about 5°C lower than the thermal melting point (T_m) 25 for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt 30 concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60°C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents,

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i.e. salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. High stringency may be attained, for example, by overnight hybridization at about 68°C in a 6X SSC solution, washing—
5 at room temperature with 6X SSC solution, followed by washing at about 68°C in a 6X SSC solution then in a 0.6X SSX solution.

Hybridization with moderate stringency may be
10 attained, for example, by: 1) filter pre-hybridizing and hybridizing with a solution of 3X sodium chloride, sodium citrate (SSC), 50% formamide, 0.1M Tris buffer at pH 7.5, 5X Denhardt's solution; 2) pre-hybridization at 37°C for 4 hours; 3) hybridization at 37°C with amount of labeled
15 probe equal to 3,000,000 cpm total for 16 hours; 4) wash in 2X SSC and 0.1% SDS solution; 5) wash 4X for 1 minute each at room temperature and 4X at 60°C for 30 minutes each; and 6) dry and expose to film.

The phrase "selectively hybridizing to" refers to a
20 nucleic acid molecule that hybridizes, duplexes or binds only to a particular target DNA or RNA sequence when the target sequences are present in a preparation of total cellular DNA or RNA. By selectively hybridizing it is meant that a nucleic acid molecule binds to a given
25 target in a manner that is detectable in a different manner from non-target sequence under moderate, or more preferably under high, stringency conditions of hybridization. "Complementary" or "target" nucleic acid sequences refer to those nucleic acid sequences which
30 selectively hybridize to a nucleic acid molecule. Proper annealing conditions depend, for example, upon a nucleic acid molecule's length, base composition, and the number of mismatches and their position on the molecule, and must often be determined empirically. For discussions of

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nucleic acid molecule (probe) design and annealing conditions, see, for example, Sambrook et al. 1989.

It will be readily understood by those skilled in the art and it is intended here, that when reference is made to particular sequence listings, such reference includes sequences which substantially correspond to its complementary sequence and those described including allowances for minor sequencing errors, single base changes, deletions, substitutions and the like, such that any such sequence variation corresponds to the nucleic acid sequence of the signal peptide or other peptide/protein to which the relevant sequence listing relates.

The DNA molecules of the subject invention also include DNA molecules coding for protein analogs, fragments or derivatives of the protein which differ from naturally-occurring forms (the naturally-occurring protein) in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues, and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the protein) and which share the signal property of the naturally-occurring form. These molecules include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily expressed vectors.

As used herein, a "peptide" refers to an amino acid sequence of three to one hundred amino acids, and

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therefore an isolated peptide that comprises an amino acid sequence is not intended to cover amino acid sequences of greater than 100 amino acids. Preferably, the peptides that can be identified and used in

5 accordance with the subject invention (whether they be mimotope or anti-mimotope peptides) are less than 50 amino acids in length, and more preferably the peptides are five to 20 amino acids in length or 20-40 amino acids in length.

10 The peptides can contain any naturally-occurring or non-naturally-occurring amino acids, including the D-form of the amino acids, amino acid derivatives and amino acid mimics, so long as the desired function and activity of the peptide is maintained. The choice of including an

15 (L)- or a (D)-amino acid in the peptides depends, in part, on the desired characteristics of the peptide. For example, the incorporation of one or more (D)-amino acids can confer increased stability on the peptide and can allow a peptide to remain active in the body for an
20 extended period of time. The incorporation of one or more (D)-amino acids can also increase or decrease the pharmacological activity of the peptide.

The peptides may also be cyclized, since cyclization may provide the peptides with superior properties over
25 their linear counterparts.

As used herein, the terms "amino acid mimic" and "mimetic" mean an amino acid analog or non-amino acid moiety that has the same or similar functional characteristic of a given amino acid. For instance, an
30 amino acid mimic of a hydrophobic amino acid is one which is non-polar and retains hydrophobicity, generally by way of containing an aliphatic chemical group. By way of further example, an arginine mimic can be an analog of arginine which contains a side chain having a positive

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charge at physiological pH, as is characteristic of the guanidinium side chain reactive group of arginine.

In addition, modifications to the peptide backbone and peptide bonds thereof are also encompassed within the 5 scope of amino acid mimic or mimetic. Such modifications-- can be made to the amino acid, derivative thereof, non-amino acid moiety or the peptide either before or after the amino acid, derivative thereof or non-amino acid moiety is incorporated into the peptide. What is 10 critical is that such modifications mimic the peptide backbone and bonds which make up the same and have substantially the same spacial arrangement and distance as is typical for traditional peptide bonds and backbones. An example of one such modification is the 15 reduction of the carbonyl(s) of the amide peptide backbone to an amine. A number of reagents are available and well known for the reduction of amides to amines such as those disclosed in Wann et al., JOC, 46:257 (1981) and Raucher et al., Tetrahedron. Lett., 21:14061 (1980). An 20 amino acid mimic is, therefor, an organic molecule that retains the similar amino acid pharmacophore groups as is present in the corresponding amino acid and which exhibits substantially the same spatial arrangement between functional groups.

25 The substitution of amino acids by non-naturally occurring amino acids and amino acid mimics as described above can enhance the overall activity or properties of an individual peptide based on the modifications to the backbone or side chain functionalities. For example, 30 these types of alterations to the specifically described amino acid substituents and exemplified peptides can enhance the peptide's stability to enzymatic breakdown and increase biological activity. Modifications to the

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peptide backbone similarly can add stability and enhance activity.

One skilled in the art, using the above sequences or formulae, can easily synthesize the peptides. Standard procedures for preparing synthetic peptides are well known in the art. The novel peptides can be synthesized using: the solid phase peptide synthesis (SPPS) method of Merrifield (J. Am. Chem. Soc., 85:2149 (1964)) or modifications of SPPS; or, the peptides can be synthesized using standard solution methods well known in the art (see, for example, Bodanzsky, M., Principles of Peptide Synthesis, 2nd revised ed., Springer-Verlag (1988 and 1993)). Alternatively, simultaneous multiple peptide synthesis (SMPS) techniques well known in the art can be used. Peptides prepared by the method of Merrifield can be synthesized using an automated peptide synthesizer such as the Applied Biosystems 431A-01 Peptide Synthesizer (Mountain View, Calif.) or using the manual peptide synthesis technique described by Houghten, Proc. Natl. Acad. Sci., USA 82:5131 (1985).

With these definitions in mind, the subject invention provides an isolated nucleic acid molecule encoding a pancreatic T-type calcium channel. The nucleic acid molecule can be deoxyribonucleic acid (DNA) or ribonucleic acid (RNA, including messenger RNA or mRNA), genomic or recombinant, biologically isolated or synthetic.

The DNA molecule can be a cDNA molecule, which is a DNA copy of a messenger RNA (mRNA) encoding the channel.

An example of such a pancreatic T-type calcium channel is the rat pancreatic T-type calcium channel encoded by the nucleotide sequence as shown in SEQ ID NO:1. The amino acid sequence encoded by this nucleotide sequence is shown in SEQ ID NO:2.

The invention also provides an antisense nucleic acid molecule that is complementary to at least a portion of the mRNA encoding the pancreatic T-type calcium channel. Antisense nucleic acid molecules can be RNA or 5 single-stranded DNA, and can be complementary to the entire mRNA molecule encoding the channel (i.e. of the same nucleotide length as the entire molecule). It may be desirable, however, to work with a shorter molecule. In this instance, the antisense molecule can be 10 complementary to a portion of the entire mRNA molecule encoding the channel. These shorter antisense molecules are capable of hybridizing to the mRNA encoding the entire molecule, and preferably consist of about twenty to about one hundred nucleotides. These antisense 15 molecules can be used to reduce levels of pancreatic T-type calcium channel, by introducing into cells an RNA or single-stranded DNA molecule that is complementary to at least a portion of the mRNA of the channel (i.e. by introducing an antisense molecule). The antisense 20 molecule can base-pair with the mRNA of the channel, preventing translation of the mRNA into protein. Thus, an antisense molecule to the channel can prevent translation of mRNA encoding the channel into a functional channel protein. It may be desirable to place 25 the antisense molecule downstream and under the control or the insulin promoter, so that the antisense will prevent translation of mRNA encoding the T type calcium channel only in islet cells of the pancreas (not affecting brain or heart T type calcium channels). It 30 should also be apparent that 100% prevention of T type calcium channel is not desirable, since a minimal basal Ca^{2+} level is required to be maintained by the T type calcium channel.

More particularly, an antisense molecule complementary to at least a portion of mRNA encoding a pancreatic T-type calcium channel can be used to decrease expression of a functional channel. A cell with a first 5 level of expression of a functional pancreatic T-type calcium channel is selected, and then the antisense molecule is introduced into the cell. The antisense molecule blocks expression of functional pancreatic T-type calcium channel, resulting in a second level of 10 expression of a functional pancreatic T-type calcium channel in the cell. The second level is less than the initial first level.

Antisense molecules can be introduced into cells by any suitable means. In one embodiment, the antisense RNA 15 molecule is injected directly into the cellular cytoplasm, where the RNA interferes with translation. A vector may also be used for introduction of the antisense molecule into a cell. Such vectors include various plasmid and viral vectors. For a general discussion of 20 antisense molecules and their use, see Han et al. 1991 and Rossi 1995.

The invention further provides a special category of antisense RNA molecules, known as ribozymes, having recognition sequences complementary to specific regions 25 of the mRNA encoding the pancreatic T-type calcium channel. Ribozymes not only complex with target sequences via complementary antisense sequences but also catalyze the hydrolysis, or cleavage, of the template mRNA molecule. Examples, which are not intended to be 30 limiting, of suitable regions of the mRNA template to be targeted by ribozymes are any of the homologous regions identified by comparing the various T-type calcium channels, and particularly pancreatic β -cell T-type channels.

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Expression of a ribozyme in a cell can inhibit gene expression (such as the expression of a pancreatic T-type calcium channel). More particularly, a ribozyme having a recognition sequence complementary to a region of a mRNA encoding a pancreatic T-type calcium channel can be used — to decrease expression of pancreatic T-type calcium channel. A cell with a first level of expression of pancreatic T-type calcium channel is selected, and then the ribozyme is introduced into the cell. The ribozyme in the cell decreases expression of pancreatic T-type calcium channel in the cell, because mRNA encoding the pancreatic T-type calcium channel is cleaved and cannot be translated.

Ribozymes can be introduced into cells by any suitable means. In one embodiment, the ribozyme is injected directly into the cellular cytoplasm, where the ribozyme cleaves the mRNA and thereby interferes with translation. A vector may be used for introduction of the ribozyme into a cell. Such vectors include various plasmid and viral vectors (note that the DNA encoding the ribozyme does not need to be "incorporated" into the genome of the host cell; it could be expressed in a host cell infected by a viral vector, with the vector expressing the ribozyme, for instance). For a general discussion of ribozymes and their use, see Sarver et al. 1990, Chrisey et al. 1991, Rossi et al. 1992, and Christoffersen et al. 1995.

The nucleic acid molecules of the subject invention can be expressed in suitable host cells using conventional techniques. Any suitable host and/or vector system can be used to express the pancreatic T-type calcium channel. For in vitro expression, Xenopus oocytes are preferred. For in vivo expression, the most suitable host cell is a pancreatic β -cell.

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Techniques for introducing the nucleic acid molecules into the host cells may involve the use of expression vectors which comprise the nucleic acid molecules. These expression vectors (such as plasmids 5 and viruses; viruses including bacteriophage) can then be used to introduce the nucleic acid molecules into suitable host cells. For example, DNA encoding the pancreatic T-type calcium channel can be injected into the nucleus of a host cell or transformed into the host 10 cell using a suitable vector, or mRNA encoding the pancreatic T-type calcium channel can be injected directly into the host cell, in order to obtain expression of pancreatic T-type calcium channel in the host cell.

15 Various methods are known in the art for introducing nucleic acid molecules into host cells. One method is microinjection, in which DNA is injected directly into the nucleus of cells through fine glass needles (or RNA is injected directly into the cytoplasm of cells).

20 Alternatively, DNA can be incubated with an inert carbohydrate polymer (dextran) to which a positively charged chemical group (DEAE, for diethylaminoethyl) has been coupled. The DNA sticks to the DEAE-dextran via its negatively charged phosphate groups. These large DNA- 25 containing particles stick in turn to the surfaces of cells, which are thought to take them in by a process known as endocytosis. Some of the DNA evades destruction in the cytoplasm of the cell and escapes to the nucleus, where it can be transcribed into RNA like any other gene 30 in the cell. In another method, cells efficiently take in DNA in the form of a precipitate with calcium phosphate. In electroporation, cells are placed in a solution containing DNA and subjected to a brief electrical pulse that causes holes to open transiently in

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their membranes. DNA enters through the holes directly into the cytoplasm, bypassing the endocytotic vesicles through which they pass in the DEAE-dextran and calcium phosphate procedures. DNA can also be incorporated into 5 artificial lipid vesicles, liposomes, which fuse with the—cell membrane, delivering their contents directly into the cytoplasm. In an even more direct approach, DNA is absorbed to the surface of tungsten microparticles and fired into cells with a device resembling a shotgun.

10 Several of these methods, microinjection, electroporation, and liposome fusion, have been adapted to introduce proteins into cells. For review, see Mannino and Gould-Fogerite 1988, Shigekawa and Dower 1988, Capecchi 1980, and Klein et al. 1987.

15 Further methods for introducing nucleic acid molecules into cells involve the use of viral vectors. One such virus widely used for protein production is an insect virus, baculovirus. For a review of baculovirus vectors, see Miller (1989). Various viral vectors have 20 also been used to transform mammalian cells, such as bacteriophage, vaccinia virus, adenovirus, and retrovirus.

As indicated, some of these methods of transforming a cell require the use of an intermediate plasmid vector. 25 U.S. Patent No. 4,237,224 to Cohen and Boyer describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and 30 replicated in unicellular cultures including prokaryotic organisms and eucaryotic cells grown in tissue culture. The DNA sequences are cloned into the plasmid vector using standard cloning procedures known in the art, as described by Sambrook et al. (1989).

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Host cells into which the nucleic acid encoding the pancreatic T-type calcium channel has been introduced can be used to produce (i.e. to functionally express) the pancreatic T-type calcium channel. The function of the 5 encoded pancreatic T-type calcium channel can be assayed — according to methods known in the art (Wang et al. 1996).

Having identified the nucleic acid molecules encoding pancreatic T-type calcium channels and methods for expressing the pancreatic T-type calcium channels 10 encoded thereby, the invention further provides a method of screening a substance (for example, a compound or inhibitor) for the ability of the substance to modify T-type calcium channel function. The method comprises introducing a nucleic acid molecule encoding the 15 pancreatic T-type calcium channel into a host cell, and expressing the pancreatic T-type calcium channel encoded by the molecule in the host cell. The cell is then exposed to a substance and evaluated to determine if the substance modifies the function of the T-type calcium 20 channel. From this evaluation, substances effective in altering the function of the T-type calcium channel can be found. Such agents may be, for example, calcium channel inhibitors, agonists, or antagonists (for example, mibepradil and mibepradil analogues, amiloride, 25 NiCl₂, antisense molecules, and second messengers).

The evaluation of the cell to determine if the substance modifies the function of the T-type calcium channel can be by any means known in the art. The evaluation can comprise the direct monitoring of 30 expression of T-type calcium channel in the host cell, or the evaluation can be indirect and comprise the monitoring of calcium transport by the channel (such as by the methods disclosed by Wang et al. 1996).

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The nucleic acid molecules of the subject invention can be used either as probes or for the design of primers to obtain DNA encoding other pancreatic T-type calcium channels by either cloning and colony/plaque

- 5 hybridization or amplification using the polymerase chain-reaction (PCR).

Specific probes derived from SEQ ID NO:1 can be employed to identify colonies or plaques containing cloned DNA encoding a member of the pancreatic T-type 10 calcium channel family using known methods (see Sambrook et al. 1989). One skilled in the art will recognize that by employing such probes under high stringency conditions (for example, hybridization at 42°C with 5X SSPC and 50% formamide, washing at 50-65°C with 0.5X SSPC), sequences 15 having regions which are greater than 90% homologous or identical to the probe can be obtained. Sequences with lower percent homology or identity to the probe, which also encode pancreatic T-type calcium channels, can be obtained by lowering the stringency of hybridization and 20 washing (e.g., by reducing the hybridization and wash temperatures or reducing the amount of formamide employed).

More particularly, in one embodiment, the method comprises selection of a DNA molecule encoding a 25 pancreatic T-type calcium channel, or a fragment thereof, the DNA molecule having a nucleotide sequence as shown in SEQ ID NO:1, and designing an oligonucleotide probe for pancreatic T-type calcium channel based on SEQ ID NO:1. A genomic or cDNA library of an organism is then probed 30 with the oligonucleotide probe, and clones are obtained from the library that are recognized by the oligonucleotide probe so as to obtain DNA encoding another pancreatic T-type calcium channel.

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Specific primers derived from SEQ ID NO:1 can be used in PCR to amplify a DNA sequence encoding a member of the pancreatic T-type calcium channel family using known methods (see Innis et al. 1990). One skilled in 5 the art will recognize that by employing such primers under high stringency conditions (for example, annealing at 50-60°C, depending on the length and specific nucleotide content of the primers employed), sequences having regions greater than 75% homologous or identical 10 to the primers will be amplified.

More particularly, in a further embodiment the method comprises selection of a DNA molecule encoding pancreatic T-type calcium channel, or a fragment thereof, the DNA molecule having a nucleotide sequence as shown in 15 SEQ ID NO:1, designing degenerate oligonucleotide primers based on regions of SEQ ID NO:1, and employing such primers in the polymerase chain reaction using as a template a DNA sample to be screened for the presence of pancreatic T-type calcium channel-encoding sequences. 20 The resulting PCR products can be isolated and sequenced to identify DNA fragments that encode polypeptide sequences corresponding to the targeted region of pancreatic T-type calcium channel.

Various modifications of the nucleic acid and amino 25 acid sequences disclosed herein are covered by the subject invention. These varied sequences still encode a functional pancreatic T-type calcium channel. The invention thus further provides an isolated nucleic acid molecule encoding a pancreatic T-type calcium channel, 30 the nucleic acid molecule encoding a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence as shown in SEQ ID NO:2. In further embodiments, the first amino acid sequence has at least

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95%, 96%, 97%, 98%, or 99% amino acid identity to SEQ ID NO:2.

The invention further provides an isolated DNA oligomer capable of hybridizing to the nucleic acid molecule encoding pancreatic T-type calcium channel according to the subject invention. Such oligomers can be used as probes in a method of detecting the presence of pancreatic T-type calcium channel in a sample. More particularly, a sample can be contacted with the DNA oligomer and the DNA oligomer will hybridize to any pancreatic T-type calcium channel present in the sample, forming a complex therewith. The complex can then be detected, thereby detecting presence of pancreatic T-type calcium channel in the sample.

15 The complex can be detected using methods known in the art. Preferably, the DNA oligomer is labeled with a detectable marker so that detection of the marker after the DNA oligomer hybridizes to any pancreatic T-type calcium channel in the sample (wherein non-hybridized DNA 20 oligomer has been washed away) is detection of the complex. Detection of the complex indicates the presence of pancreatic T-type calcium channel in the sample. As will be readily apparent to those skilled in the art, such a method could also be used quantitatively to assess 25 the amount of pancreatic T-type calcium channel in a sample.

For detection, the oligomers can be labeled with, for example, a radioactive isotope, biotin, an element opaque to X-rays, or a paramagnetic ion. Radioactive 30 isotopes are commonly used and are well known to those skilled in the art. Representative examples include indium-111, technetium-99m, and iodine-123. Biotin is a standard label which would allow detection of the biotin labeled oligomer with avidin. Paramagnetic ions are also

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commonly used and include, for example, chelated metal ions of chromium (III), manganese (II), and iron (III).

When using such labels, the labeled DNA oligomer can be imaged using methods known to those skilled in the art.

5 Such imaging methods include, but are not limited to, x-ray, CAT scan, PET scan, NMRI, and fluoroscopy. Other suitable labels include enzymatic labels (horseradish peroxidase, alkaline phosphatase, etc.) and fluorescent labels (such as FITC or rhodamine, etc.).

10 The invention further provides an isolated pancreatic T-type calcium channel protein. The protein is preferably encoded by a nucleotide sequence as shown in SEQ ID NO:1. The protein preferably has an amino acid sequence as shown in SEQ ID NO:2. Further provided is an

15 isolated pancreatic T-type calcium channel protein encoded by a first amino acid sequence having at least 90% amino acid identity to a second amino acid sequence, the second amino acid sequence as shown in SEQ ID NO:2.

In further embodiments, the first amino acid sequence has
20 at least 95%, 96%, 97%, 98%, or 99% amino acid identity to SEQ ID NO:2.

The pancreatic T-type calcium channel molecule of the subject invention can include a leader sequence for targeting of the pancreatic T-type calcium channel
25 protein to the desired part of a cell.

It should be readily apparent to those skilled in the art that a met residue may need to be added to the amino terminal of the amino acid sequence of the mature pancreatic T-type calcium channel protein (i.e., added to
30 SEQ ID NO:2) or an ATG added to the 5' end of the nucleotide sequence (i.e., added to SEQ ID NO:1), in order to express the channel in a host cell. The met version of the mature channel is thus specifically

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intended to be covered by reference to SEQ ID NO:1 or SEQ ID NO:2.

The invention further provides an antibody or fragment thereof specific for the pancreatic T-type calcium channel of the subject invention. Antibodies of the subject invention include polyclonal antibodies and monoclonal antibodies capable of binding to the pancreatic T-type calcium channel, as well as fragments of these antibodies, and humanized forms. Humanized forms of the antibodies of the subject invention may be generated using one of the procedures known in the art such as chimerization. Fragments of the antibodies of the present invention include, but are not limited to, the Fab, the F(ab')₂, and the Fc fragments.

15 The invention also provides hybridomas which are capable of producing the above-described antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing polyclonal and 20 monoclonal antibodies as well as hybridomas capable of producing the desired antibody are well known in the art (see Campbell 1984 and St. Groth et al. 1980). Any animal (mouse, rabbit, etc.) which is known to produce antibodies can be immunized with the antigenic pancreatic 25 T-type calcium channel (or an antigenic fragment thereof). Methods for immunization are well known in the art. Such methods include subcutaneous or intraperitoneal injection of the protein. One skilled in the art will recognize that the amount of the protein used for immunization will vary based on the animal which is immunized, the antigenicity of the protein, and the site of injection.

The protein which is used as an immunogen may be modified or administered in an adjuvant in order to

increase the protein's antigenicity. Methods of increasing the antigenicity of a protein are well known in the art and include, but are not limited to, coupling the antigen with a heterologous protein (such as a 5 globulin or beta-galactosidase) or through the inclusion — of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/O-Ag 15 myeloma cells, and allowed to become 10 monoclonal antibody producing hybridoma cells.

Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, 15 western blot analysis, or radioimmunoassay (Lutz et al. 1988).

Hybridomas secreting the desired antibodies are cloned and the class and subclass are determined using procedures known in the art (Campbell 1984).

20 For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

The present invention further provides the above- 25 described antibodies in detectably labeled form.

Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, etc.), fluorescent labels (such as 30 FITC or rhodamine, etc.), paramagnetic atoms, etc.

Procedures for accomplishing such labeling are well known in the art, for example see Sternberger et al. 1970, Bayer et al. 1979, Engval et al. 1972, and Goding 1976.

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The labeled antibodies or fragments thereof of the present invention can be used for in vitro, in vivo, and in situ assays to identify cells or tissues which express pancreatic T-type calcium channel, to identify samples 5 containing pancreatic T-type calcium channel, or to detect the presence of pancreatic T-type calcium channel in a sample. More particularly, the antibodies or fragments thereof can thus be used to detect the presence of pancreatic T-type calcium channel in a sample, by 10 contacting the sample with the antibody or fragment thereof. The antibody or fragment thereof binds to any pancreatic T-type calcium channel present in the sample, forming a complex therewith. The complex can then be detected, thereby detecting the presence of pancreatic T- 15 type calcium channel in the sample. As will be readily apparent to those skilled in the art, such a method could also be used quantitatively to assess the amount of pancreatic T-type calcium channel in a sample. As should also be readily apparent, such an antibody can also be 20 used to decrease levels of functional T type calcium channels, by blocking the channel. Such antibodies can therefore be used in the methods of the subject invention to modify levels of functional T type calcium channels in pancreatic beta cells.

25 Further provided is a composition comprising the pancreatic T-type calcium channel protein and a compatible carrier.

In the methods of the invention, tissues or cells are contacted with or exposed to the composition of the 30 subject invention or a compound. In the context of this invention, to "contact" tissues or cells with or to "expose" tissues or cells to a composition or compound means to add the composition or compound, usually in a liquid carrier, to a cell suspension or tissue sample,

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either in vitro or ex vivo, or to administer the composition or compound to cells or tissues within an animal (including humans).

For therapeutics, methods of modifying insulin secretion by pancreatic beta cells, methods of treating type II diabetes, methods of modifying basal calcium levels in cells, methods of modifying the action potential of L type calcium channels in cells, methods of modifying pancreatic beta cell death, methods of modifying pancreatic beta cell proliferation, and methods of modifying calcium influx through L type calcium channels in cells, each of the methods comprising modifying levels of functional T type calcium channels in the cells, are provided. The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill in the art. In general, for therapeutics, a patient suspected of needing such therapy is given a composition in accordance with the invention, commonly in a pharmaceutically acceptable carrier, in amounts and for periods which will vary depending upon the nature of the particular disease, its severity and the patient's overall condition. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal, transdermal), oral or parenteral. Parenteral administration includes intravenous drip or infusion, subcutaneous, intraperitoneal or intramuscular injection, pulmonary administration, e.g., by inhalation or insufflation, or intrathecal or intraventricular administration.

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Formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders.

Conventional pharmaceutical carriers, aqueous, powder or 5 oily bases, thickeners and the like may be necessary or - desirable. Coated condoms, gloves and the like may also be useful.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non- 10 aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

Compositions for parenteral, intrathecal or intraventricular administration may include sterile 15 aqueous solutions which may also contain buffers, diluents and other suitable additives.

In addition to such pharmaceutical carriers, cationic lipids may be included in the formulation to facilitate uptake. One such composition shown to 20 facilitate uptake is LIPOFECTIN (BRL, Bethesda MD).

Dosing is dependent on severity and responsiveness of the condition to be treated, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is 25 achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body.

Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates.

Optimum dosages may vary depending on the relative 30 potency of individual compositions, and can generally be calculated based on IC₅₀'s or EC₅₀'s in in vitro and in vivo animal studies. For example, given the molecular weight of compound (derived from oligonucleotide sequence and/or chemical structure) and an effective dose such as

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an IC₅₀, for example (derived experimentally), a dose in mg/kg is routinely calculated.

The methods of the subject invention are based on the discovery that regulation of T type calcium channels directly modifies basal calcium levels in cells, which in turn regulates L type calcium channel activity, which in turn regulates insulin secretion and cell death, which in turn treats type II diabetes. The methods of the subject invention are further based on the discovery that regulation of T type calcium channels directly affects basal and glucose-induced insulin secretion.

T type calcium channels belong to the family of low voltage activated calcium channels. Modifying (increasing or decreasing) "levels" of functional T type calcium channels refers to modifying expression of the T type calcium channel gene, modifying activity of the T type calcium channel such as by inhibiting the function of the channel, and/or modifying the formation of active membrane-spanning T type calcium channels. As used herein, "functional" refers to the synthesis and any necessary post-translational processing of a calcium channel molecule in a cell so that the channel is inserted properly in the cell membrane and is capable of conducting calcium ions in accordance with a low voltage activated channel.

The invention thus provides a method of modifying insulin secretion by pancreatic beta cells, the method comprising modifying levels of T type calcium channels in the pancreatic beta cells.

Levels of T type calcium channels in the pancreatic beta cells can be modified by various methods, at the gene and protein and "functional calcium channel" levels. In one embodiment, the levels are modified by modifying T type calcium channel gene expression of the T type

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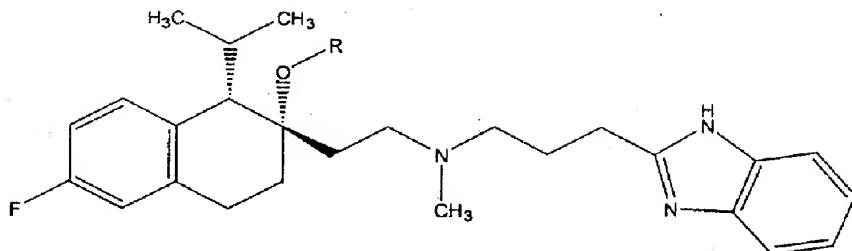
calcium channel in the cells. This can be accomplished by exposing the cells to a compound which modifies T type calcium channel gene expression of the calcium channel. The compound could be, for example, an antisense 5 oligonucleotide targeted to the T type calcium channel gene. In a similar embodiment, the compound which modifies T type calcium channel gene expression of the T type calcium channel could be a ribozyme.

Other methods for modifying T type calcium channel 10 gene expression could also involve site-directed mutagenesis of the T type calcium channel gene to prevent expression of the T type calcium channel, or various gene therapy techniques.

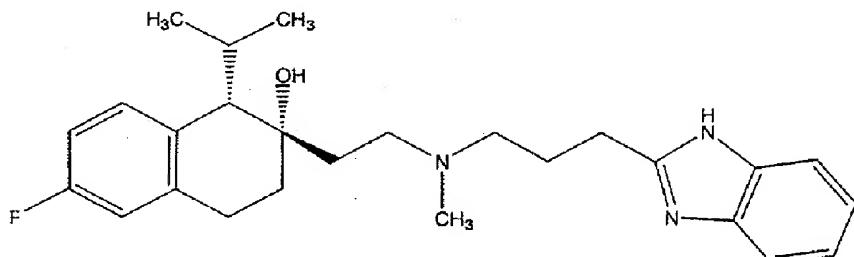
Levels, in particular activity, of T type calcium 15 channels in the cell can also be modified by exposing the cells to an inhibitor of the T type calcium channel. Such inhibitors include, for example, mibepradil, mibepradil analogs, amiloride, NiCl_2 , and second 20 messengers which regulate activity of the T type calcium channels. Other inhibitors of the T type calcium channel could also readily be identified by screening methods (including the method described above). In addition to chemical inhibitors, peptide inhibitors could also be identified with screening methods (for example, using 25 phage display libraries and other peptide screening methods).

"Mibepradil analogs", as used herein are meant to include compounds having the formula:

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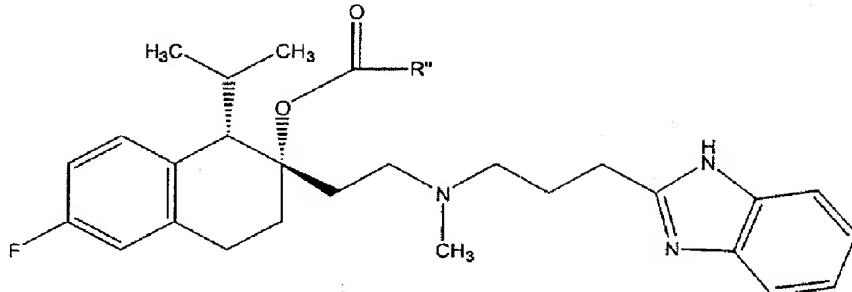


wherein R is hydrogen, alkyl, or a moiety having the formula C(O)R', where R' is alkyl or aryl. In the above 5 formulae, alkyl is meant to include linear alkyls, particularly C₁-C₁₂ linear alkyls (e.g., methyl, ethyl, n-propyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, and the like), branched alkyls, particularly C₁-C₁₂ branched alkyls (e.g., isobutyl, isopentyl, neopentyl, 10 hex-2-yl, hex-3-yl, hept-2-yl, hept-3-yl, and the like), and cycloalkyls, particularly C₁-C₈ cycloalkyls (e.g., cyclopentyl, cyclohexyl, cycloheptyl, 4-methylcyclohexyl, and the like). These alkyl groups can be substituted or unsubstituted. When substituted, suitable substituents 15 include, for example, aryl groups, halogen atoms, hydroxy groups, alkoxy groups, carboxylic acid groups, amine groups, and the like, as well as combinations of these substituents. Mibepradil analogs which are particularly well suited to blocking (inhibiting) the activity of T- 20 type calcium channels but not blocking the activity of L-type calcium channels are those having the formula:



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and those having the formula:



- 5 in which R" is an unsubstituted alkyl group or a substituted alkyl group which does not contain an alkoxy substituent. "Mibefradil analogs" are also meant to include compounds having the above formulae which are substituted at other positions in the structure, for
 10 example, on the benzimidazole phenyl moiety, at a benzimidazole nitrogen, at other positions of the tetrahydronaphthyl ring, etc. Also included within the meaning of "mibefradil analogs" are compounds having the above formulae in which the F is replaced with another
 15 substituent, such as another halogen. Also included within the meaning of "mibefradil analogs" are compounds having the above formulae in which the amine methyl group or the isopropyl group or both are replaced with other substituents, such as other alkyl moieties.
- 20 Additionally, "mibefradil analogs" are meant to include those compounds which are generically described and/or specifically disclosed in U.S. Patent No. 4,808,605, which is hereby incorporated by reference. Further, "mibefradil analogs" are meant to include
 25 pharmaceutically acceptable salts of the derivatives described above. Illustrative pharmaceutically acceptable salts are salts formed with hydrochloric acid, hydrobromic acid, nitric acid, sulphuric acid, phosphoric acid, citric acid, formic acid, maleic acid, acetic acid,

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succinic acid, tartaric acid, methanesulphonic acid, p-toluenesulphonic, and the like.

Mibepradil analogs can be made by following the general procedures described in, for example, U.S. Patent 5 Nos. 4,808,605, 5,910,606, 5,892,055, 5,811,557, 5,811,556, and 5,808,088, each of which is hereby incorporated by reference.

Levels of T type calcium channels in the cell can also be modified by exposing the cells to a compound 10 which interferes with membrane T type calcium channel formation.

Levels of functional T type calcium channel could also be modified by use of molecules which bind to transcription regulators of the T type calcium channel 15 gene (such as the promoter region of the gene).

The invention further provides a method of treating type II diabetes in a subject (human or animal), the method comprising administering to the subject an amount of a compound effective to modify levels of T type 20 calcium channels in the pancreatic beta cells of the subject. As above, the compound may modify levels of T type calcium channels by modifying T type calcium channel gene expression of the calcium channel, or by inhibiting the T type calcium channel, or by interfering with 25 membrane T type calcium channel formation.

In the context of this invention "modulation" or "modifying" means either inhibition or stimulation. This modulation can be measured in ways which are routine in the art, for example by Northern blot assay of mRNA 30 expression, Western blot assay of protein expression, or calcium channel activity assay.

The compounds and/or inhibitors used in the methods of the subject invention encompass any pharmaceutically acceptable salts, esters, or salts of such esters, or any

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other compound/inhibitor which, upon administration to an animal including a human, is capable of providing (directly or indirectly) the biologically active metabolite or residue thereof. Accordingly, for example, 5 the disclosure is also drawn to prodrugs and pharmaceutically acceptable salts of the compounds and/or inhibitors used in the subject invention, pharmaceutically acceptable salts of such prodrugs, and other bioequivalents.

10 In regard to prodrugs, the compounds and/or inhibitors for use in the invention may additionally or alternatively be prepared to be delivered in a prodrug form. The term prodrug indicates a therapeutic agent that is prepared in an inactive form that is converted to 15 an active form (i.e., drug) within the body or cells thereof by the action of endogenous enzymes or other chemicals and/or conditions.

In regard to pharmaceutically acceptable salts, the term pharmaceutically acceptable salts refers to 20 physiologically and pharmaceutically acceptable salts of the compounds and/or inhibitors used in the subject invention: i.e., salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

25 Drugs, such as peptide drugs, which inhibit the T type calcium channel or which interfere with functional T type calcium channel formation can be identified by other methods also. For example, a monoclonal antibody can be prepared which specifically hybridizes to the T type 30 calcium channel, thereby interfering with activity and/or channel formation. Once a monoclonal antibody which specifically hybridizes to the T type calcium channel is identified, the monoclonal (which is itself a compound or inhibitor which can be used in the subject invention) can

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be used to identify peptides capable of mimicking the inhibitory activity of the monoclonal antibody. One such method utilizes the development of epitope libraries and biopanning of bacteriophage libraries. Briefly, attempts
5 to define the binding sites for various monoclonal antibodies have led to the development of epitope libraries. Parmley and Smith developed a bacteriophage expression vector that could display foreign epitopes on its surface (Parmley, S.F. & Smith, G.P., Gene 73:305-318
10 (1988)). This vector could be used to construct large collections of bacteriophage which could include virtually all possible sequences of a short (e.g. six-amino-acid) peptide. They also developed biopanning,
15 which is a method for affinity-purifying phage displaying foreign epitopes using a specific antibody (see Parmley, S.F. & Smith, G.P., Gene 73:305-318 (1988); Cwirla, S.E., et al., Proc Natl Acad Sci USA 87:6378-6382 (1990);
Scott, J.K. & Smith, G.P., Science 249:386-390 (1990); Christian, R.B., et al., J Mol Biol 227:711-718 (1992);
20 Smith, G.P. & Scott, J.K., Methods in Enzymology 217:228-257 (1993)).

After the development of epitope libraries, Smith et al. then suggested that it should be possible to use the bacteriophage expression vector and biopanning technique
25 of Parmley and Smith to identify epitopes from all possible sequences of a given length. This led to the idea of identifying peptide ligands for antibodies by biopanning epitope libraries, which could then be used in vaccine design, epitope mapping, the identification of genes, and many other applications (Parmley, S.F. &
30 Smith, G.P., Gene 73:305-318 (1988); Scott, J.K., Trends in Biochem Sci 17:241-245 (1992)).

Using epitope libraries and biopanning, researchers searching for epitope sequences found instead peptide

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sequences which mimicked the epitope, i.e., sequences which did not identify a continuous linear native sequence or necessarily occur at all within a natural protein sequence. These mimicking peptides are called 5 mimotopes. In this manner, mimotopes of various binding sites/proteins have been found.

The sequences of these mimotopes, by definition, do not identify a continuous linear native sequence or necessarily occur in any way in a naturally-occurring 10 molecule, i.e. a naturally occurring protein. The sequences of the mimotopes merely form a peptide which functionally mimics a binding site on a naturally-occurring protein.

Many of these mimotopes are short peptides. The 15 availability of short peptides which can be readily synthesized in large amounts and which can mimic naturally-occurring sequences (i.e. binding sites) offers great potential application.

Using this technique, mimotopes to a monoclonal 20 antibody that recognizes T type calcium channels can be identified. The sequences of these mimotopes represent short peptides which can then be used in various ways, for example as peptide drugs that bind to T type calcium channels and decrease the activity of T type calcium 25 channels. Once the sequence of the mimotope is determined, the peptide drugs can be chemically synthesized.

MATERIALS AND METHODS

30 Cell Culture - INS-1 cells were cultured in RPMI 1640 medium containing 10% FBS, 25 U/ml penicillin, 25 mg/ml streptomycin and 50 μ M mercaptoethanol in an atmosphere of 5% CO₂ in air, at 37°C for 2-5 days before recording.

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Islet cell preparation - Pancreases of Sprague-Dawley rats (Charles River Laboratory, Wilmington, MA) were removed after intrapancreatic perfusion with 2 ml of Hanks' solution (Gibco BRL, Grand Island, NY) containing collagenase (4 mg/ml, Boehringer - Mannheim, Indianapolis, IN), DNase I (10 µg/ml, Sigma, St. Louis, MO), CaCl₂ (1.28 mM) and bovine serum albumin (1 mg/ml, Gibco BRL). The pancreatic tissue was incubated at 37°C for 20 min and then washed five times with enzyme-free Hanks' solution. Islets were picked up and treated with 0.1% pancreatin (Sigma) for five minutes at 37°C. Single cells were obtained by triturating the islets with plastic pipette tips and then they were transferred into 35 mm culture dishes. Cells were cultured in RPMI 1640 medium (Gibco BRL) containing 5 mM glucose, 10% FBS and P/S at 37°C, 5% CO₂ for 2-5 days before experiments.

Isolation of RNA - Total RNA was isolated from cultured INS-1 cells and from various freshly excised rat tissues by the guanidinium isothiocyanate/phenol procedure (Chomczynsk and Sacchi 1987). Poly-A RNA was isolated from total RNA by two successive passes over an oligo (dT)-cellulose spin column (Ambion, Austin, TX).

Cloning of cDNA Encoding α₁ Subunit of T-type Ca²⁺ channel in INS-1 - First strand cDNA was prepared using 2 µg of INS-1 cell mRNA and M-MLV reverse transcriptase (Gibco BRL) with the poly-dT primers. The first 433 bp DNA fragment of the channel was deduced with PCR using the degenerate primers (forward) (SEQ ID NO:6) 5'-TNGC (A/C/T) ATGGAG (C/A) GNCC (C/T) -3' and (backward) (SEQ ID NO:7) 5'-CTT (C/G/T) CCCTTGAA (G/C) A (G/A) CTG -3' based on conserved voltage-dependent Ca²⁺ channel α₁ subunit sequences in domain III. Using the Marathon™ cDNA Amplification Kit (Clontech, Palo Alto, CA), the 3'- and

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5'- rapid amplifications of cDNA end-PCR (RACE-PCR) were performed to obtain the entire gene of the α_1 subunit of the channel. For the 5'-RACE-PCR, the forward primer was an adapter primer, the backward primer was (SEQ ID NO:8) 5'-CCGCTGTCGGAGACCATGGAGACC-3'; for the 3'-RACE, the forward primer was (SEQ ID NO:9) 5'-AGCGGCCAAAATTGACCCCCACAG-3' and the backward primer was poly-dT. The RT-PCR products were subcloned into pT-Adv Vector (Clontech) and dideoxynucleotide sequencing assay 10 was performed with a dsDNA Cycle Sequencing System (Gibco BRL).

Tissue distribution - The gene expression of T-type Ca^{2+} channels deduced from β -cells was examined in rat brain, heart, kidney, and liver using an RT-PCR assay. 15 The primers used for the RT-PCR were (SEQ ID NO:10) 5'-GAAGATGCGAGTGGACAG-3' (forward) and (SEQ ID NO:11) 5'-CTGTGGCGATGGTCACTG-3' (backward). The PCR products were detected by agarose gel electrophoresis on a 1% gel.

Genome walking - The genome walker library 20 (Clontech) was used as a template in nested PCR reactions with gene-specific primers (GSP) and the adapter primers (AP) provided with the kit. The first PCR reaction was carried out in 5 tubes, each having a total volume of 50 μl : 5 μl 10X PCR reaction buffer, 1 μl dNTP (10 mM each), 2.2 μl Mg(OAc)₂ (25 mM), 1 μl AP1 (10 μM), 1 μl GSP1, 1 μl Advantage Genomic Polymerase Mix (50X), and 37.8 μl water. The following two-step cycle parameters were used: (Step 1) 7 cycles of denaturing at 94°C for 25 sec., annealing and extension at 72°C for 4 min. (Step 2) 30 32 cycles of denaturing at 94°C for 25 sec., annealing and extension at 67°C for 4 min. After the second step cycle, the samples were held at 67°C for 4 min. The second PCR reaction was carried out under the reaction condition similar to the first PCR reaction except using

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AP2, GSP2. In addition, the templates used were 1 μ l of 1:50 dilution of each primary PCR reaction. The two step cycles were similar to the first PCR reaction except 5 cycles at the first step and 22 cycles at the second
5 step.

Oocyte electrophysiology - cRNA transcripts were synthesized from BssH II linearized pT-Adv cDNA templates using T7 RNA polymerase (Ambion). Defolliculated Xenopus laevis were injected with 25 ng pT-Adv cRNA. Three to 10 five days after injection, two-electrode voltage-clamp recording was performed using a Warner OC-725C amplifier (Warner Instrument Corp., Hamden, CT). Data were acquired and analyzed with Pulse/PulseFit software (HEKA, Lambrecht/Pfalz, Germany). The bath solution contained 15 the following: 40 mM Ca(OH)₂, 50 mM NaOH, 2 mM TEA-Cl, 1mM KOH, 0.1 mM EDTA and 5 mM HEPES, adjusted to pH 7.4 with methanesulphonate. Boltzmann fits were calculated using Prism (GraphPad). Results are presented as mean \pm s.d. unless otherwise stated.

20 β -cell Electrophysiological recording - The whole-cell recordings were carried out by the standard "giga-seal" patch clamp technique (Hamill et al.). The whole-cell recording pipettes were made of hemocapillaries (Warner), pulled by a two-stage puller 25 (PC-10, Narishige International, New York, NY), and heat polished with a microforge (MF200-1, World Precision Instruments, Sarasota, FL) before use. Pipette resistance was in the range of 2-5 M Ω in the internal solution. The recordings were performed at room temperature (22-25°C).
20 Currents were recorded using an EPC-9 patch-clamp amplifier (HEKA) and filtered at 2.9 kHz. Data were acquired with Pulse/PulseFit software (HEKA). Voltage-dependent currents were corrected for linear leak

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and residual capacitance by using an on-line P/n subtraction paradigm.

Drugs - Mibefradil ((1S,2S)-2-[2-[[3-(2-Benzimidazolyl)propyl]methyl-amino]ethyl]-6-fluoro-
5 1,2,3,4-tetrahydro-1-isopropyl-2-naphthyl methoxy-acetate - dihydrochloride) was kindly provided by Dr. J.-P. Clozel (Hoffmann LaRoche, Basel, Switzerland), and can be synthesized according to the methods disclosed in U.S. Patent Nos. 5,892,055, 5,811,557, 5,811,556, and
10 5,808,088. U.S. Patent No. 4,808,605 describes mibefradil compounds suitable for use in the subject invention.

The free alcohol Des-methoxyacetyl mibefradil (1S,2S)-2-[2-[[3-(2-Benzimidazolyl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphthyl hydroxy hydrochloride) was prepared by alkaline hydrolysis: 14.2 mg mibefradil hydrochloride was dissolved in 4 ml methanol + 1 ml 10 N aqueous sodium hydroxide mixture (5 mM was the final concentration of
20 mibefradil). The solution was warmed in a boiling water bath for 10 min. The reaction was followed by mass spectrometry. Upon completion of the hydrolysis, as determined from the mass spectra, the solution was neutralized with 5 M aqueous hydrochloric acid. The
25 slight loss of methanol that occurred by evaporation during the reaction was corrected by adding water to keep the total volume of 5 ml.

Solutions - The extracellular solution used in whole-cell Ca²⁺ current recording contained (in mM): 10
30 CaCl₂, 110 tetraethylammonium-Cl (TEA-Cl), 10 CsCl, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 40 sucrose, 0.5 3,4-diaminopyridine, pH 7.3. The intracellular solution contained (in mM): 130 N-methyl-D-glucamine, 20 EGTA (free acid), 5 bis (2-aminophenoxy)

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ethane-N, N, N', N'-tetraacetate (BAPTA), 10 HEPES, 6 MgCl₂, 4 Ca(OH)₂, pH was adjusted to 7.4 with methanesulfonate. 2 mM Mg-ATP was included in the pipette solution to minimize rundown of L-type Ca²⁺ currents. For 5 Perforated-patch recording, the extracellular solution contained (in mM): 26 Sucrose, 30 TEA-Cl, 10 HEPES, 5 KCl, 2 CaCl₂, MgCl₂, pH 7.3. The pipette solution contained (in mM): 65 CsOH, 65 CsMS, 20 sucrose, 10 HEPES, 10 MgCl₂, 1 Ca(OH)₂, pH 7.4.

10 Mass Spectrometric Analysis - A VG 70-250 SEQ instrument (VG Analytical, Manchaster, UK) was used with fast atom bombardment (FAB) ionization mode to obtain mass spectra of the mibepradil and dm-mibepradil. Cultured INS-1 cells were treated with 20 μM mibepradil 15 for various lengths of time under each experimental condition. The cell pellets were collected after washing three times with PBS and resuspended in 0.5 ml media for mass spectrometric analysis. For a 50 μl cell sample, 20 μl internal standard solution (40 μM verapamil, MW:454) 20 and 5 μl glycerol was added, and 4 μl of this mixture was used for FAB-MS. Several positive ion spectra were recorded in the mass range m/z 750-100 at a mass resolution of 1000, and a scan speed of 2 second/decade. For mibepradil, m/z 496 was the dominant ion (M+H)⁺ 25 accompanied with a less intense sodiated molecular ion m/z 518. The concentrations of the mibepradil and hydrolyzed mibepradil were obtained by comparing the intensities of m/z 496 and 424 were to the intensity of m/z 455. For calibration, a standard solution of 50 μM 30 drug was subjected to mass spectrometric analysis.

Separation of cytosolic and membrane components - After washing out mibepradil from the bath solution, the cells were collected and the membranes were broken down by vortexing the cells in a solution containing 5% acetic

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acid/CH₃CN. The mixture was then spun and the supernatant collected and defined as non-membrane associated components. Pellets were re-suspended in 5 x volume of NaOH (10 N):methanol (1:7) solution at 37°C for 5 min.

- 5 The mixture was neutralized with 0.5 M HCl and spun down.— The remaining pellet and the supernatant were collected separately.

Statistics - All data is presented as mean ± s.d. and the student's t-test was used to calculate p values
10 where given.

EXAMPLE I

Identification and Cloning of a Pancreatic T-type Calcium Channel

15 The subject invention provides a cDNA encoding a T-type Ca²⁺ channel α_1 subunit derived from the rat insulin secreting cell line, INS-1, which has been identified and sequenced. The sequence of the cDNA indicates a protein composed of 2288 amino acids (SEQ ID NO:2), sharing 96.3% identity to the neuronal T-type Ca²⁺ channel α_1 subunit (α_1G). The transmembrane domains of the protein are highly conserved but the isoform contains three distinct regions as well as 10 single amino acid substitutions in other regions. Sequencing rat genomic
20 DNA revealed that this is an alternative splice isoform of α_1G . Using specific primers and reverse transcription polymerase chain reaction (RT-PCR) it was demonstrated that both splice variants are expressed in rat islets. The isoform deduced from INS-1 was also expressed in
25 brain, neonatal heart and kidney. Functional expression of this α_1G isoform in *Xenopus* oocytes generated low-voltage activated Ca²⁺ currents. These results provide the molecular biological basis for studies of function of
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T-type Ca^{2+} channels in β -cells where these channels play critical roles in diabetes.

The cloning and tissue distribution of an isoform of the T-type Ca^{2+} channel ($\alpha_1\text{G-INS}$) derived from the rat 5 insulin-secreting cell line, INS-1 (Asfari et al. 1992), — is described further below.

Based on the conserved amino acid sequence comprising the six transmembrane segments in repeat III of the previously cloned α_1 -subunit (Stea et al. 1995), 10 degenerate primers were designed to deduce the cDNA sequence of voltage-dependent Ca^{2+} channel from INS-1 which expresses a high level of T-type Ca^{2+} current (Bhattacharjee et al. 1997). A 433 base pair (bp) DNA fragment was obtained. The rapid amplification of cDNA 15 ends (RACE) strategy was then used to obtain the entire sequence of the channel. The full length cDNA (SEQ ID NO:1) encodes a protein containing 2288 amino acids (SEQ ID NO:2).

The T-type Ca^{2+} channel gene deduced from β -cells 20 shares 96.3% amino acid identity with $\alpha_1\text{G}$, the neuronal isoform of T-type Ca^{2+} channel (Perez-Reyes et al. 1998). The four intramolecular homologous transmembrane domains of β -cell T-type Ca^{2+} channel α_1 subunit are identical (except glycine 1667) to $\alpha_1\text{G}$, with each repeat containing 25 six putative membrane-spanning regions (S1-S6) and a pore-forming region (P-loop). The other highly conserved region is located at the intracellular loop between repeat I and II, where a section of histidine-rich chain is present in the β -cell derived T-type Ca^{2+} channel gene 30 as well as in neuronal and cardiac T-type Ca^{2+} channel genes. This structure in the loop_{I-II} has not been observed in the protein sequences of known high voltage activated Ca^{2+} channels.

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In addition to the single amino acids that differ from α_1G , the T-type Ca^{2+} channel gene derived from β -cells contains three unique regions that differ from the amino acid sequence of α_1G . These regions are located
5 at the N-terminal amino acids (aa1-34 of SEQ ID NO:2), — intracellular loop I_{III-IV} (aa971-994 of SEQ ID NO:2) and intracellular loop L_{III-IV} (aa1570-1588 of SEQ ID NO:2).

Although the amino acid sequence of the deduced channel is entirely different from the α_1G in the
10 N-terminal region (aa1-34 of SEQ ID NO:2), the nucleotide sequences at this region are almost identical except for 4 single nucleotide insertions which are shown in Fig. 1A. These four single nucleotide insertions determine a different start codon as well as those of the amino acid
15 sequences.

To resolve the relationship between the T-type Ca^{2+} channel isoform deduced from INS-1 and α_1G , a section of Sprague-Dawley rat genomic DNA sequence containing the introns and exons between 4845 and 5256 was identified.
20 As shown in Fig. 1B, an exon was found that encodes the α_1G fragment SKEKQMA (SEQ ID NO:5) as well as an exon that encodes fragment 4869-4922 of the INS-1 variant. This region also contains 8.5 kilobases (kb) of intron sequence. Thus, the T-type Ca^{2+} channel α_1 subunit cloned
25 from INS-1 and α_1G are alternative splice isoforms of the same gene.

The genomic DNA sequence was also used to examine the two nucleotide discrepancy between the α_1G cDNA and the isoform cloned from INS-1. The data show that the
30 genomic nucleotide sequence encoding amino acid 1667 is GGC (glycine), which is the same as the cDNA of α_1 subunit cloned from INS-1 and the corresponding residue in α_1H , but is different from α_1G (GCG, alanine). Also of note, there are nine additional single amino acid substitutions

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in the isoform deduced from INS-1 as compared to the α_1G . Six correspond to the amino acids found in the analogous position of α_1H : cysteine 1088, glycine 1667, alanine 1700, aspartic acid 1735, threonine 1812, and leucine 5 1813.

In regard to tissue distribution of T-type Ca^{2+} channels deduced from β -cells and from neurons, expression of the β -cell T-type Ca^{2+} channel was found in rat brain, heart and kidney, but was absent from liver. 10 Both α_1G and the splice form were detected in rat islets and INS-1 cell preparations using RT-PCR. No α_1H was detected.

Functional expression of the T-type Ca^{2+} channels deduced from β -cells has been conducted in Xenopus 15 oocytes using a double-electrode voltage-clamp method. In a solution containing 40 mM Ca^{2+} , a family of current traces representing T-type Ca^{2+} current characteristics were obtained (Fig. 2A). The current slowly activated at -40 mV and peaked at -10 mV. The analysis of time 20 constants of activation and inactivation are shown in Fig. 2B. The voltage-dependent activation (Fig. 2C) and steady-state inactivation (Fig. 2D) were fitted with Boltzmann equation. The calculated $V_{1/2}$'s were -23.8 mV and -45.6 mV for activation and inactivation, 25 respectively; and k 's were 5.3 and -6.0 for activation and inactivation, respectively.

The nucleotide cDNA (SEQ ID NO:1) and amino acid (SEQ ID NO:2) sequences of rat pancreatic T-type calcium channel were determined. SEQ ID NO:3 is the nucleotide 30 sequence beyond the coding region, while SEQ ID NO:4 includes SEQ ID NO:2.

EXAMPLE II**Characterization of the T type Calcium Channel
in Relation to Diabetes**

Glucose stimulated insulin release is Ca^{2+} dependent process, involving closure of the ATP-sensitive potassium-channels, depolarization and opening of the voltage-dependent Ca^{2+} channels. At glucose concentrations below 3 mM, which do not elicit insulin secretion, β -cells are electrically silent with a resting membrane potential of about -70 mV. Raising external glucose produces a slow depolarization, the extent dependent upon the glucose concentration. At glucose levels which elicit insulin release (>7 mM) depolarization is sufficient to reach the threshold potential (-50 mV) at which electrical activity is initiated.

A simple model for glucose-stimulated insulin secretion is summarized in Fig. 12. The resting membrane potential of β -cells is principally determined by the activity of the K-ATP channel. When plasma glucose rises, its uptake and rate of metabolism by β -cells are stimulated. As a consequence, the intracellular ATP (or ATP:ADP ratio) increases which leads to the closure of K-ATP channels and membrane depolarization. This results in the activation of voltage dependent Ca^{2+} channels (T-type and L-type) and the initiation of electrical activity. The increased calcium influx leads to a rise in $[\text{Ca}^{2+}]_i$ and consequently insulin secretion.

Rat and human pancreatic β -cells are equipped with L-type and T-type Ca^{2+} channels. The physiological function of T-type Ca^{2+} channels in β -cells insulin-secretion has been demonstrated. These channels facilitate exocytosis by enhancing electrical activity in these cells. L-type and T-type Ca^{2+} channels, under normal conditions, work in concert promoting the rise in

[Ca²⁺]_i during glucose-stimulated insulin secretion. In β-cells, over-expressed T-type Ca²⁺ channels are, at least in part, responsible for the hyper-responsiveness of insulin secretion to non-glucose depolarizing stimuli in 5 GK rat, and in rat with NIDDM induced by neonatal injection of streptozotocin. However, over-expressed T-type calcium channels over time will ultimately lead to an elevation of basal Ca²⁺ through its window current properties. Therefore, there is a dual effect of T-type 10 Ca²⁺ channels in β-cells depending upon channel number and membrane potential.

Pharmacologically antagonizing T-type calcium channels is an appropriate treatment protocol for alleviating both insulin resistance and enhancement of 15 insulin secretion in NIDDM patients.

NIDDM pathogenesis is complex and the disease progression occurs in phases. An enhanced β-cell responsiveness provokes and initiates the disease process. It is unclear as to what the actual enhanced 20 activity is and what the triggering mechanisms are for this first phase. It may be an increased secretory response or an increase in β-cell mass. However, there is clearly an enhancement of β-cell activity detected by both basal and postprandial elevated insulin levels 25 denoted as hyperinsulinemia. Consequently, a resulting insulin resistance occurs, phase II, particularly in insulin responsive tissues (muscle, liver, kidney, fat) that function to reduce glucose levels in the blood. A decrease in insulin sensitivity will account for an 30 increase in blood glucose, causing the β-cells to secrete even more insulin to compensate and because of this vicious cycle, full blown NIDDM, marked by an inevitable defect in insulin release, hyperglycemia and insulin

resistance, will characterize the final stage of the disease process.

Each phase of the disease may be characterized by an alteration in $[Ca^{2+}]_i$, and each phase can be treated by a 5 T-type calcium channel antagonist. The electrical β -cell— is equipped with two types of voltage-dependent calcium channels, L-type and T-type calcium channels. L-type calcium channels, activated at high voltages, having large unitary conductance, and dihydropyridine-sensitive, 10 are considered the major pipeline for calcium influx into the β -cell (especially at high voltage depolarization). T-type calcium channels, activated at low voltages, with small unitary conductance, and dihydropyridine-insensitive, are important for maintaining basal $[Ca^{2+}]_i$ 15 (Fig. 8), as well as enhancing electrical activity during cell depolarization. T-type calcium channels normally facilitate insulin secretion in β -cells by enhancing cell electrical activity. This modulatory function of T-type calcium channels in insulin secretion is significant 20 during phase I prior to onset of diabetes. Antagonizing these T-type calcium channels will decrease β -cell hyperresponsiveness and consequent hyperinsulinemia arresting the pathogenic pathways that lead to NIDDM.

If hyperinsulinemia and associated insulin 25 resistance has already occurred, a T-type calcium channel blocker is still the appropriate treatment protocol. The insulin responsive tissues, those that are primarily responsible for taking up glucose for re-establishing euglycemia, have elevated basal $[Ca^{2+}]_i$ during 30 hyperinsulinemic conditions. Indeed, it is the elevated basal $[Ca^{2+}]_i$ that precipitates the decrease in insulin sensitivity of these tissues and it is now known that most of these insulin responsive tissues express T-type calcium channels. A T-type calcium channel blocker will

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reduce the basal $[Ca^{2+}]_i$, and alleviate the decreased insulin sensitivity.

Once NIDDM has manifested, it is characterized by altered glucose metabolism, a result of abnormal glucose stimulus-secretion responsiveness of β -cells. β -cell desensitization to glucose is the principal secretory defect of NIDDM. L-type and T-type calcium channels, under normal conditions, work in concert promoting the rise in $[Ca^{2+}]_i$ during glucose-stimulated insulin secretion. In NIDDM, this partnership is broken and the necessary rise in $[Ca^{2+}]_i$ for insulin secretion is compromised.

The data herein indicates that L-type calcium channels are finely regulated by basal calcium levels (Figs. 9A-9D). A very small rise in basal calcium will substantially decrease the L-type calcium current and severely reduce the depolarization-induced rise in $[Ca^{2+}]_i$ (Figs. 10 and 11). The data herein also suggests that T-type calcium channels are a primary regulator of resting basal $[Ca^{2+}]_i$ in β -cells. Furthermore, the negative feedback regulation of T-type calcium channels by elevated $[Ca^{2+}]_i$ is absent (Figs. 9A-9D). It is under circumstances of enhanced T-type calcium current activity as seen in the GK rat model of NIDDM and in the neonate streptozotocin-induced diabetes model, that basal $[Ca^{2+}]_i$ is elevated, and a defect in the glucose-stimulated insulin secretion is observed. Simply reducing the basal calcium influx by pharmacological intervention, in situations of enhanced T-type calcium channel expression, may reduce basal $[Ca^{2+}]_i$ in β -cells (Fig. 8) and alleviate the $[Ca^{2+}]_i$ -induced inhibition of L-type calcium channels.

There is a clear link between $[Ca^{2+}]_i$ and diabetes. A primary abnormality in $[Ca^{2+}]_i$ handling by cells is the defect initiating parallel impairments in insulin

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secretion and insulin action, as well as initiating diabetic complications. Consequent metabolic derangements may further aggravate alterations in $[Ca^{2+}]_i$ homeostasis, creating a relentless cycle leading to progressive deterioration in the overall health of the diabetic patient. Pharmacological agents that regulate $[Ca^{2+}]_i$ homeostasis are thus appropriate therapeutic measures. The use of T-type calcium channel blockers will thus effectively treat and perhaps cure diabetes mellitus.

EXAMPLE III

Pharmacology of Mibepradil Action

It has been shown that mibepradil has a potent inhibitory effect on T-type Ca^{2+} current in vascular smooth muscle cells. The data herein demonstrates that, in conventional whole cell patch clamp configuration, mibepradil also blocks T-type Ca^{2+} current in pancreatic β -cells. Mibepradil (1 μ M) had been administered in the recording chamber at time zero (Fig. 13), the control (no drug) showed "run down". This figure shows that T-type Ca^{2+} current is more sensitive to mibepradil than the L-type Ca^{2+} current in pancreatic β -cells.

The blockade of T-type Ca^{2+} channels in β -cells with mibepradil is reversible. Fig. 14 demonstrates the reversibility of blockade of T-type Ca^{2+} currents by mibepradil. In these experiments, a very little volume of mibepradil or $NiCl_2$, was delivered near the recording cell. The drug then diffused away from the cell. The final concentration in the chamber was 1 nM. This experiment shows the inhibitory effect of mibepradil on T-type Ca^{2+} current in pancreatic β -cells results from reversible interaction between the drug and the channel protein.

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In β -cells, T-type Ca^{2+} channels could mediate a small, but sustained, Ca^{2+} influx by means of their unique "window" current at voltages near resting membrane potentials. Like other voltage-regulated channels, T-type Ca^{2+} channels are opened and closed depending upon the potentials across the cell membranes. This voltage dependency is illustrated in Fig. 15. The activation and inactivation curves represent the percentage of the channels in either open or closed states over a range of voltages. Unlike most of the voltage-dependent Na^+ channels or L-type Ca^{2+} channels, the activation and inactivation curves of T-type Ca^{2+} channels overlap at the certain range of low voltages (i.e. window). In other words, there is a small portion of T-type Ca^{2+} channels that stay in non-inactivated states in this voltage range. The data in Fig. 15 was obtained from experiments conducted under 10 mM external Ca^{2+} condition, which shifted the window current about 10 mV toward positive voltage due to the surface charge effects of divalent ions on the channels.

The existence of a window current provides a negative feedback regulation of $[\text{Ca}^{2+}]_i$ in β -cells. When cells are under an unhealthy condition, they may be slightly depolarized to activate window current, which elevates the basal $[\text{Ca}^{2+}]_i$ to protect the cells from further Ca^{2+} influx through the L-type Ca^{2+} channels. This process is reversible if the membrane potential is reset to the normal resting potential (-70 mV).

Mibepradil regulates basal $[\text{Ca}^{2+}]_i$ in pancreatic β -cells:

The data herein demonstrates the roles of T-type calcium currents in modulating basal $[\text{Ca}^{2+}]_i$ in INS-1 cells (Fig. 8). $[\text{Ca}^{2+}]_i$ was directly measured by the ratio of fluorescence excitations at Ca^{2+} -bound (380 nm)

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to unbound (340 nm), and then the ratio was converted to the calcium concentration. The bath solution contained 10 mM NaCl, 4 mM KCl, 2 mM CaCl₂, and 2 mM MgCl₂. In a single cell exhibiting fluctuating basal [Ca²⁺]_i with an average value near 150 nM, administering 1 μM mibepradil into the chamber immediately lowered the basal calcium. This data shows the T-type calcium currents participate in regulating the mean basal [Ca²⁺]_i in cultured β-cells.

10 Mibepradil regulates basal insulin secretion:

The activation of T-type Ca²⁺ channel at low voltage near the resting membrane potential of pancreatic β-cells suggests that the channels are responsible for the Ca²⁺ influx required for insulin secretion under non-stimulus conditions. The NIT-1 cell line was chosen to demonstrate the effect of mibepradil on the basal insulin secretion. NIT-1 is a cell line derived from the β-cell of non-obese-diabetic mouse. This cell line expressed high levels of T-type Ca²⁺ current. The data herein shows that 5 μM mibepradil reduced the basal insulin secretion to less than 40% of control (Fig. 17), indicating this drug is able to lower the high basal insulin secretion level seen during the earlier stage of NIDDM.

25 Spontaneous elevation of [Ca²⁺]_i:

To demonstrate that T-type Ca²⁺ channels play an important role in calcium entry under non-stimulatory conditions, and therefore regulate basal [Ca²⁺]_i, spontaneous elevations of intracellular free calcium concentration was detected with the Fluo-3 AM fluorescent imaging method. NIT-1 cells were cultured in medium containing 3.3 mM glucose and preloaded with 2.5 μM Fluo-3 AM. The numbers of spontaneous calcium elevated cells were counted and compared to the total cells being used

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for a 10 minute observation period. 10 μM NiCl₂ inhibited 90% of spontaneous elevation of basal Ca²⁺.

The cellular mechanism of the spontaneous elevation of intracellular Ca²⁺ was investigated with the epifluorescence measurement method. Some INS-1 cells were observed to exhibit transient spontaneous elevations of [Ca²⁺]_i, "Calcium spikes", under non-stimulatory conditions. The Role of T-type Ca²⁺ channels in this spontaneous process was examined as well. In a single cell with spontaneous calcium spike activity (Fig. 17), NiCl₂ (30 μM) reduced the frequency of spontaneous calcium spikes immediately. This result suggests that either the T-type Ca²⁺ channels alone or together with the L-type Ca²⁺ channels are responsible for the transient spontaneous elevation of [Ca²⁺]_i, under conditions where no glucose is present. These spontaneous calcium spikes may contribute to basal insulin secretion and control of basal [Ca²⁺]_i.

However, neither mibepradil nor NiCl₂ exhibited their effect on basal [Ca²⁺]_i in all of the β -cells. It was observed that only those cells which had relatively higher initial basal [Ca²⁺]_i will respond to the T-type Ca²⁺ channel antagonists (Fig. 18). Whereas those cells with lower initial basal [Ca²⁺]_i had no or less response to the T type Ca²⁺ channel antagonists. This result indicates that T type Ca²⁺ channel antagonists may selectively act on the cells with high basal [Ca²⁺]_i and bring it back to normal, by inhibiting the window current.

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EXAMPLE IV

Action on Pancreatic β -cells

T type Ca²⁺ may play two pathological roles in NIDDM. At the earlier stage, the NIDDM patients exhibit

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hyperinsulinemia and β -cell hyperexcitability. This may, at least in part, be due to increased activity of T type Ca^{2+} channel in β -cells. At the more developed NIDDM stage, over-expressed T type Ca^{2+} channel and membrane

5 depolarization resulted from reduced generation of ATP, and may set up a window current in β -cells that causes chronic elevation of basal Ca^{2+} in the β -cells. The elevated basal Ca^{2+} will reduce the L-type Ca^{2+} activity and glucose induced insulin secretion.

10 It has been shown that mibefradil prevented and reversed development of hyperinsulinemia in rat. This result indicates this drug is a valuable candidate for the treatment of earlier stage NIDDM or for preventing NIDDM in the potential patients.

15 A series of experiments were conducted with INS-1 cells to show that T type Ca^{2+} facilitated insulin secretion by enhancing the general excitability of pancreatic β -cells. Particularly, activation of T type Ca^{2+} channels will increase the firing frequency of the 20 depolarizing spikes mediated by opening L type Ca^{2+} channels (Fig. 19A). Activation of T type Ca^{2+} channel will also decrease the time of developing action potential elicited by up-threshold depolarizations (Fig. 19B).

25 To further establish that T type Ca^{2+} current enhances β -cell excitability, 100 μM NiCl_2 was administered to effectively block T type Ca^{2+} channels. In contrast to control experiments, NiCl_2 caused a delay 30 in the onset of an action potential and a decrease in number of action potentials.

To directly demonstrate the role of T type Ca^{2+} current in glucose-induced insulin secretion, INS-1 cells were incubated with 11.1 mM glucose and variable concentrations of NiCl_2 , and insulin release was measured.

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NiCl₂ reduced insulin secretion in a dose-dependent manner (Fig. 20A). On the other hand, clonal insulin secreting cells (HIT-T15, which did not consistently exhibit T type Ca²⁺ current) were not affected by NiCl₂ (Fig. 20B).

5 These results show that T type Ca²⁺ channels play an important role in β-cell excitability and antagonists of T type Ca²⁺ channels (such as NiCl₂) will effectively reduce the excitability of β-cells.

Although T type Ca²⁺ channels facilitate insulin 10 secretion by enhancing general excitability of β-cells, the function of T type Ca²⁺ channels is a double-edged sword. Under the condition of over-expressed T type Ca²⁺ channel in β-cells, the function of the window current will become dominant and result in an elevation of basal 15 Ca²⁺. High [Ca²⁺]_i may cause impairment of insulin release by inactivating L type Ca²⁺ channels.

L-type Ca²⁺ channels are partially inactivated by [Ca²⁺]_i in non-stimulus condition in β-cells:

20 Upon establishment of a whole-cell patch, within the first five minutes, the L type Ca²⁺ current "runs-up", as the magnitude of the peak current increases over time in INS-1 cells (Fig. 21). This phenomenon is a universal feature in these cells under the recording conditions 25 used. The pipette solutions contained no ATP but did contain high concentrations of the calcium chelating agents BAPTA and EGTA. When the pipette solution contained high Ca²⁺, this run-up does not occur. Instead, a rapid run down occurs. The "run-up" phenomenon is 30 likely due to calcium chelation inside the cells. T type Ca²⁺ currents do not show this effect.

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Intracellular perfusion patch clamp experiments demonstrated that basal $[Ca^{2+}]_i$ regulates L type Ca^{2+} current amplitude in INS-1 cells:

Intracellular perfusion of a solution containing 5 high Ca^{2+} (Fig. 9A) causes a substantial reduction in the L type Ca^{2+} current. L type Ca^{2+} currents were elicited by a voltage step to +10 mV from a holding potential of -80 mV. The $[Ca^{2+}]_i$ was measured directly using fura-2 ratiometric fluorescence. The effect of a high $[Ca^{2+}]_i$ 10 (272 nM) on the IV relationship is shown in Fig. 9B. Perfusioning in high $[Ca^{2+}]_i$, substantially reduces the high voltage current component, but does not affect the low current component. The high $[Ca^{2+}]_i$ caused a shift in peak current to negative voltages, and Ca^{2+} currents were 15 enhanced at negative voltages. This effect seemed to result in a potentiation of the T type Ca^{2+} current (Fig. 9D). Slow deactivating T type Ca^{2+} currents showed a shift in activation upon perfusion of high $[Ca^{2+}]_i$. This may account for the shift in IV. Various concentrations 20 of $[Ca^{2+}]_i$ regulated the activity of L type Ca^{2+} channels (Fig. 9C). Perfusioning a low $[Ca^{2+}]_i$ from an existing high $[Ca^{2+}]_i$ (632 nM to 0 nM) caused an increase in the L type Ca^{2+} current over time, however perfusing in high $[Ca^{2+}]_i$ 25 (0 nM to 272 nM and 0 nM to 632 nM) inhibits the L type Ca^{2+} current over time. The levels of $[Ca^{2+}]_i$ therefore have regulatory effects on both the L type Ca^{2+} current and T type Ca^{2+} current, with $[Ca^{2+}]_i$ having significant feedback regulation on the L type Ca^{2+} current.

30 Effect of basal $[Ca^{2+}]_i$ on Ca^{2+} influx:

The effect of basal $[Ca^{2+}]_i$ on Ca^{2+} influx was examined using the Ca^{2+} dye indicator fura-2 and fluorescence measurements. Voltage-dependent Ca^{2+} influx in a single cell was obtained by perfusion of an

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osmotically balanced solution containing 50 mM KCl into the recording chamber. Voltage-dependent increases in $[Ca^{2+}]_i$ occur primarily through nifedipine sensitive Ca^{2+} channels. The resting basal $[Ca^{2+}]_i$ in INS-1 cells was approximately 60-80 nM under the experimental conditions.— $[Ca^{2+}]_i$ was determined by a standard curve obtained from a fura-2 calcium imaging kit (Molecule Probes). The empirical K_d obtained for calcium binding to fura-2 in the system was 296 ± 20 nM. When basal $[Ca^{2+}]_i$ remains low, subsequent voltage stimulation with 50 KCl induces rapid and large calcium influx into the cell and these calcium changes are stereotyped upon repetitive stimulation when basal calcium is restored (Fig. 10). In this experiment, following the 50 KCl depolarization, the cell was repolarized by perfusion of the original 5 mM KCl solution. After repolarization, basal $[Ca^{2+}]_i$ slowly reset and then a second 50 KCl depolarization induced a similar $[Ca^{2+}]_i$ transient. When the basal calcium is not allowed to reset, a defect in the second voltage induced calcium transient occurs (Fig. 11). In this experiment, after repolarization, the second depolarization was applied before basal $[Ca^{2+}]_i$ could return to its original value, and thus, the $[Ca^{2+}]_i$ transient is substantially reduced. These findings suggest that basal $[Ca^{2+}]_i$ plays a prominent role in the regulation of voltage dependent Ca^{2+} influx in INS-1 cells. Therefore effectors of basal $[Ca^{2+}]_i$ will have important impact on the amount of calcium that can enter the cell.

Streptozotocin induced high basal $[Ca^{2+}]_i$ inhibits KCl stimulated Ca^{2+} influx:

To reiterate the importance of basal $[Ca^{2+}]_i$ on voltage stimulated Ca^{2+} influx, basal $[Ca^{2+}]_i$ in INS-1 cells was artificially enhanced by pretreating the cells

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with the toxicant, streptozotocin. Though it is known that streptozotocin induces DNA strand breaks, it has also been shown to induce Ca^{2+} channel activity in β -cells. The data shows that pretreating cells with 5 mM streptozotocin for 1 hour, followed by 3 hour recovery, causes a two-fold increase in basal calcium (Fig. 22). These cells when stimulated by 50 KCl had reduced calcium influx compared to control cells.

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EXAMPLE V

Inhibition of T type Calcium Channel with Mibepradil Metabolite

It has been shown that mibepradil (Ro 40-5967) exerts a selective inhibitory effect on T-type Ca^{2+} currents, although at higher concentrations it can antagonize high voltage-activated Ca^{2+} currents. The action of mibepradil on Ca^{2+} channels is use- and steady state-dependent and the binding site of mibepradil on L-type Ca^{2+} channels is different from that of dihydropyridines. By using conventional whole-cell and perforated patch-clamp, mibepradil is shown to have an inhibitory effect on both T- and L-type Ca^{2+} currents in insulin-secreting cells. However, the effect on L-type Ca^{2+} currents was time-dependent and poorly reversible in perforated patch experiments. Using mass spectrometry it was demonstrated that mibepradil was trapped inside cells and furthermore, a metabolite of mibepradil was detected. Intracellular application of this metabolite selectively blocked the L-type Ca^{2+} current whereas mibepradil exerted no effect. This study shows that mibepradil permeates into cells and is hydrolyzed to a metabolite that blocks L-type Ca^{2+} channels specifically by acting at the inner side of the channel.

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Whole-cell patch clamp and a bath perfusion system were first used to establish the dose-dependent inhibition of mibepradil on both T- and L-types of Ca^{2+} currents. The T-type Ca^{2+} current was measured at -30 mV when the membrane was held at -90 mV and the L-type current was measured at +20 mV when the membrane was held at -40 mV. The currents were measured twice at each concentration of mibepradil with 2 min in between measurements. The dose dependent inhibition of T-type Ca^{2+} current is shown in Fig. 3A. The 50% inhibitory concentration (IC_{50}) was 865 nM. No time-dependent inhibition was observed. In contrast, the inhibition of L-type Ca^{2+} currents could not be fitted with a one-to-one binding curve (Fig. 3B). Administration of 1 μM mibepradil progressively reduced L-type Ca^{2+} current up to 70% of the beginning amplitude after 10 minutes ($n = 4$), which indicated that a more complicated pharmacological mechanism was involved in the action of mibepradil on the L-type Ca^{2+} currents.

A drug diffusing system was then used to test the reversibility of the antagonism of T- and L-type Ca^{2+} currents by mibepradil. Small volumes (approximately 2 μl) of drugs were delivered in close proximity to the recording cell with a quartz capillary positioned by a micromanipulator. After administration, drugs diffused throughout the entire recording chamber containing 2 ml of bath solution. This drug diffusing system was used to test the reversibility of 30 μM of NiCl_2 on the T-type Ca^{2+} currents (Fig. 4). The amplitude of T-type current was rapidly reduced to 40% and gradually returned to 80% of the initial level within 3 minutes. Using this system, it was found that the inhibition of mibepradil on the T-type Ca^{2+} current was clearly reversible. In contrast,

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the inhibition of the L-type Ca^{2+} current was poorly reversible (Fig. 4).

The poor reversibility and time-dependent inhibition of the L-type Ca^{2+} current by mibepradil suggested that 5 this drug may have an accumulation effect over time. This hypothesis was tested by applying a very low dose of mibepradil on cells and recording the L-type Ca^{2+} currents for a long time in the perforated patch-clamp configuration. As shown in Fig. 5A, after 25 minutes of 10 10 nM mibepradil administration, the relative currents were reduced to 70%, whereas the currents remained unchanged for control patches. Incubation of cells with 10 nM mibepradil for two hours resulted in further reduction of current densities as recorded by perforated 15 patches (Fig. 5B). At a concentration of 10 nM, mibepradil exhibited no long-term effect on the T-type Ca^{2+} current.

To test the hypothesis that mibepradil may permeate through the cell membrane to the cytoplasm and be trapped 20 inside cells, the presence of mibepradil was examined in cells pre-incubated with 20 μM of mibepradil using mass spectrometry. After 3 washes, mibepradil (peaked at 496 MW) was still detected in cells (Fig. 6B). The concentration of intracellular mibepradil after one 25 minute incubation was $3.18 \pm 0.78 \mu\text{M}$ ($n = 3$). The localization of mibepradil in cells was examined by measuring the concentration of mibepradil in the pellets and supernatants after lysis of the cells. Most of the 30 mibepradil (92%) was detected in the supernatants and 0% was found in the pellets after washing cells with methanol, indicating that mibepradil was trapped in the cytoplasm. In addition, a peak (MW = 423) was detected which represented a hydrolyzed metabolite of mibepradil, Des-methoxyacetyl mibepradil (dm-mibepradil), which is a

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major metabolite as documented previously (Wiltshire et al. 1992). By varying the time of pre-incubation, it was found that dm-mibefradil accumulated inside the cells in a time-dependent manner (Fig. 6A). This accumulation is 5 consistent with the concept that dm-mibefradil has lower — membrane permeability than its precursor mibefradil.

It was then tested whether or not mibefradil or dm-mibefradil inhibits L- or T-type Ca^{2+} currents from inside of cells. Both L- and T-type currents were 10 measured in the whole-cell patch clamp configuration when 1 μM of mibefradil or dm-mibefradil was included in the pipette solution. As shown in Figs. 7A and 7B, intracellular application of 1 μM mibefradil did not have inhibitory effects on either L-type or T-type Ca^{2+} 15 currents, whereas the same concentration of dm-mibefradil specifically blocked the L-type Ca^{2+} current. As the bath solution contained no drug in this series of experiments, the inhibitory effect of dm-mibefradil is considered to be acting on the inside domain of L-type Ca^{2+} channels.

20 The inhibitory effect of dm-mibefradil on T-type Ca^{2+} currents was similar to the effect of mibefradil when it was applied in the bath solution, suggesting that the methoxyacetyl group of mibefradil does not play a key role in binding to the extracellular receptor site of 25 T-type Ca^{2+} channel protein. However, this methoxyacetyl group is necessary for blocking L-type Ca^{2+} channel from the inside of cells, indicating a modification in the methoxyacetyl group of mibefradil can result in a more selective antagonist of T-type Ca^{2+} channels.

EXAMPLE VI

**LVA Ca^{2+} Current Mediates Cytokine-Induced
Pancreatic β -cell Death**

Insulin-dependent diabetes mellitus is characterized by the selective destruction of pancreatic β -cells. Chronic treatment with cytokines induced a low voltage-activated (LVA) Ca^{2+} current in mouse β -cells. The concomitant increase in the basal cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) was associated with DNA fragmentation and cell death. Antagonists of LVA Ca^{2+} channels prevented this elevation of basal $[\text{Ca}^{2+}]_i$ and DNA fragmentation, and reduced the percentage of cell death. Exposure to cytokines did not affect the profile of Ca^{2+} currents or basal $[\text{Ca}^{2+}]_i$ in glucagon-secreting α -cells. An increased Ca^{2+} signal through LVA Ca^{2+} channels may thus be a key feature in cytokine-induced β -cell destruction.

The effects of chronic cytokine treatment on the voltage-sensitive Ca^{2+} currents in primary cultured mouse islet cells was examined. After treatment with IL-1 β (25 U/ml) and IFN γ (300 U/ml) for 6 h, an LVA Ca^{2+} current was induced in these cells (Fig. 23A). This current was present in 48% of cytokine-treated mouse islet cells. No such current was observed when the cells were treated with either IL-1 β or IFN γ alone. Experiments were conducted at different times recording LVA Ca^{2+} currents induced by cytokines, and the results indicate that no further increase in current density occurs even after treatment for 48 h. This LVA current has not been observed in non-treated cells. The steady state inactivation curve of the cytokine-induced LVA Ca^{2+} currents displayed a low voltage property (Fig. 23E) similar to the inactivation curve of the LVA currents in NOD mouse islets cells. This current was also blocked by NiCl_2 (10 μM ; n = 4; Fig. 23F). It has been reported that

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low concentration of NiCl₂, selectively block LVA current, a profound increase in Ca²⁺ current density was observed over the voltages between -20 and 20 mV. These high voltage-activated Ca²⁺ currents are nifedipine sensitive
5 currents (completely blocked by 10 μM nifedipine), and the increase in this current density is similar to the increased L type Ca²⁺ current density observed after treatment of β-cells with serum from IDDM patients.

As α-cells are more resistant to the toxic effects
10 of cytokines than β-cells, the effects of cytokines on the Ca²⁺ currents in a glucagon-secreting cell line (α-TC1) was also examined. This cell line, like α-cells, is more resistant to the cytotoxic effect of cytokines.

Treatment of α-TC1 cells with IL-1β and IFNγ failed to
15 induce LVA Ca²⁺ currents and did not alter the current density (Figs. 23C and 23D). Therefore, the induction of LVA Ca²⁺ currents and increased Ca²⁺ current density observed after chronic treatment with cytokines showed specificity for β-cells.

20 LVA Ca²⁺ channels are activated at low membrane potentials. This unique feature may allow them to regular [Ca²⁺]_i under nonstimulatory conditions. Indeed, basal [Ca²⁺]_i in cytokine-treated cells was approximately 3-fold higher than in nontreated cells (Fig. 24A). This
25 increase in basal [Ca²⁺]_i was blocked by NiCl₂ (10 μM), but not by the L type Ca²⁺ channel antagonist, nifedipine (10 μM). Cytokines failed to increase basal [Ca²⁺]_i in α-TC1 cells (Fig. 24B). These results suggest that Ca²⁺ influx through LVA Ca²⁺ channels is responsible for the cytokine-
30 induced elevation in basal [Ca²⁺]_i in β-cells.

It has been shown that cytokines induce apoptosis in human pancreatic islet cells. Apoptosis is also the mode of cell death in the development of IDDM in the NOD mouse and in multiple low dose streptozotocin-induced IDDM in

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the mouse, and is involved in β -cell destruction. As a marker of apoptosis, DNA fragmentation has been reported to precede β -cell lysis.

β -TC3 cells, a mouse β -cell line, were used to demonstrate the role of LVA Ca^{2+} channels in cytokine-mediated DNA fragmentation. The LVA Ca^{2+} current density was first examined before and after cytokine treatment. The LVA Ca^{2+} current (at $V_m = -30 \text{ mV}$) in β -TC3 cells was increased from $1.86 \pm 0.33 \text{ (pA/pF; n = 30)}$ to $3.45 \pm 0.47 \text{ (pA/pF; n = 10)}$ after treatment with cytokines (25 U/ml IL-1 β , 100 U/ml IFN γ , and 100 U/ml TNF α) for 25 h. This indicates that the LVA Ca^{2+} current in β -TC3 cells is regulated by cytokines, as seen in mouse islet cells. As shown in Fig. 24, cytokine-induced DNA fragmentation displayed a ladder pattern of oligonucleosomal fragments. The three LVA Ca^{2+} channel blockers, NiCl_2 , amiloride, and mibepradil, all independently prevented cytokine-induced DNA fragmentation. In contrast, nifedipine had not inhibitory effect on DNA fragmentation induced by cytokines. This experiment has been repeated in β -TC3 cells ($n = 2$) as well as in NIT-1 cells ($n = 3$), a β -cell line derived from NOD mice, and the same results were obtained.

The function of LVA Ca^{2+} channels in cytokine-mediated cell death in β -TC3 cells was then examined. Many cells died when the medium contained 25 U/ml IL-1 β , 100 U/ml IFN γ , and 100 U/ml TNF α ; however, NiCl_2 (20 μM) effectively reduced the β -cell killing potency of cytokines in both a time- and dose-dependent manner (Figs. 25A and 25B, respectively). In contrast, nifedipine did not exhibit a protective effect. Similar results were obtained from an experiment conducted in NIT-1 cells with mibepradil, which also reduced β -cell death induced by cytokines. These results demonstrate

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that LVA Ca^{2+} channels enhance the vulnerability of β -cells to the cytotoxic effects of cytokines.

Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

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SEQUENCE LISTING

DNASTS® DNA Translation [T-INS]
 File Name : T-INS
 Range : 2 - 7286 Mode : Normal
 Codon Table : Universal

5' GAG CTG AGC TGA ACT GGC CCT CCT GGG GAC TCA GCA AGC TCT CTA GAG CCC CCC
 10 19 28 37 46 55
 E L S * T G P P G D S A S S L E P P

ACA TGC TCC CCC ACC GGG GTC CCC CGG TTG CGT GAG GAC ACC TCC TCT GAG GGG
 64 73 82 91 100 109
 T C S P T G V P R L R E D T S S E G

CTC CGC TCG CCC CTC TTC GGA CCC CCC GGG GCC CGG GCT GGC CGG AGG ATG GAC
 118 127 136 145 154 163
 L R S P L F G P P G A P A G Q R M D

GAG GAG GAT GGA GCG GGC GCC GAG GAG TCG GGA CAG CCC CGT AGC TTC ACG
 172 181 190 199 208 217
 E E E D G A G A E E S G Q P R S F T

CAG CTC AAC GAC CTG TCC GGG GCC GGG GGC CGG CAG GGG CCG GGG TCG ACG GAA
 226 235 244 253 262 271
 Q L N D L S G A G G R Q G P G S T E

AAG GAC CCG GGC AGC GCG GAC TCC GAG GCG GAG GGG CTG CCG TAC CCG GCG CTA
 280 289 298 307 316 325
 K D P G S A D S E A E G L P Y P A L

GCC CCG GTG GPT TTC TIC TAC TTG AGC CAG GAC AGC CGC CCG CGG AGC TGG TGT
 334 343 352 361 370 379
 A P V V F F Y L S Q D S R P R S W C

CTC CGC ACG GTC TGT AAC CCG TGG TTC GAG CGA GTC AGT ATG CTG GTC ATT CTT
 388 397 406 415 424 433
 L R T V C N P W F E R V S M L V I L

CTC AAC TGT GTG ACT CTG GGT ATG TTC AGG CGG TGT GAG GAC ATT GCC TGT GAC
 442 451 450 459 478 487
 L N C V T L G M F R P C E D I A C D

TCC CAG CGC TGC CGG ATC CTG CAG GCC TTC GAT GAC TTC ATC TTT GCC TTC TTT
 496 505 514 523 532 541
 S Q R C R I L Q A F D D F I F A F F

GCT GTG GAA ATG GTG GTG AAG ATG GTG GCC TTG GGC ATC TTT GGG AAG AAA TGT
 550 559 568 577 586 595
 A V E M V V K M V A L G I F G K K C

TAC CTG GGA GAC ACT TGG AAC CGG CTT GAC TTT TTC ATT GTC ATT GCA GGG ATG
 604 613 622 631 640 649
 Y L G D T W N R L D F F I V I A G M

Start of
56Q ID No: 1
36Q ID No: 2
(coding regions)

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DNASIS DNA Translation [T-INS]

658	667	676	685	694	703
CTG GAG TAT TCG CTG GAC CTG CAG AAC GTC AGC TTC TCC GCA GTC AGG ACA GTC					
L E Y S L D L Q N V S F S A V R T V					
712	721	730	739	748	757
CGT GTG CTG CGA CCG CTC AGG GCC ATT AAC CGG GTG CCC AGC ATG CGC ATT CTC					
R V L R P L R A I N R V P S M R I L					
766	775	784	793	802	811
GTC ACA TTA CTG CTG GAC ACC TTG CCT ATG CTG GGC AAC GTC CTG CTG CTC TGT					
V T L L L D T L P M L G N V L L L C					
820	829	838	847	856	865
TTC TTC GTC TTT TTC ATC TTT GGC ATC GTG GGC GTC CAG CTG TGG GCA GGA CTG					
F F V F F I F G I V G V Q L W A G L					
874	883	892	901	910	919
CTT CGC AAC CGA TGC TTC CTC CCC GAG AAC TTC AGC CTC CCC CTG AGC GTG GAC					
L R N R C F L P E N F S L P L S V D					
928	937	946	955	964	973
CTG GAG CCT TAT TAC CAG ACA GAG AAT GAG GAC GAG AGC CCC TTC ATC TGC TCT					
L E P Y Y Q T E N E D E S P F I C S					
982	991	1000	1009	1018	1027
CAG CCT CGG GAG AAT GGC ATG AGA TCC TGC AGG ACT GTG CCC ACA CTG CGT GGG					
Q P R E N G M R S C R S V P T L R G					
1036	1045	1054	1063	1072	1081
GAA GGC GGT GGT GGC CCA CCC TGC AGT CTG GAC TAT GAG ACC TAT AAC AGT TCC					
E G G G P P C S L D Y E T Y N S S					
1090	1099	1108	1117	1126	1135
AGC AAC ACC ACC TGT GTC AAC TGG AAC CAG TAC TAT ACC AAC TGC TCT GCG GGC					
S N T T C V N W N Q Y Y T N C S A G					
1144	1153	1162	1171	1180	1189
GAG CAC AAC CCC TTC AAA GGC GCC ATC AAC TTT GAC AAC ATT GGC TAT GCC TGG					
E H N P F K G A I N F D N I G Y A W					
1198	1207	1216	1225	1234	1243
ATC GCC ATC TTC CAG GTC ATC ACA CTG GAG GGC TGG GTC GAC ATC ATG TAC TTC					
I A I F Q V I T L E G W V D I M Y F					
1252	1261	1270	1279	1288	1297
GTA ATG GAC CCT CAC TCC TTC TAC AAC TTC ATC TAC TTC ATT CTT CTC ATC ATC					
V M D A H S F Y N F I Y F I L L I I					
1306	1315	1324	1333	1342	1351
GTG GGC TCC TTC TTC ATG ATC AAC CTG TGC CTG GTG GTG ATT GCC ACG CAG TTC					
V G S F F M I N L C L V V I A T Q F					

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DNASIS DNA Translation [T-INS]

1360	1369	1378	1387	1396	1405
TCC GAG ACC AAA CAG CGG GAG AGT CAG CTG ATG CGG GAG CAG CGT GTA CGA TTC					
S E T K Q R E S Q L M R E Q R V R F					
1414	1423	1432	1441	1450	1459
CTG TCC AAT GCT AGC ACC CTG GCA AGC TTC TCT GAG CCA GCC AGC TGC TAT GAG					
L S N A S T L A S F S E P G S C Y E					
1468	1477	1486	1495	1504	1513
GAG CTA CTC AAG TAC CTG GTG TAC ATC CTC CGA AAA GCA GCC CGA AGG CTG GCC					
E L L K Y L V Y I L R K A A R R L A					
1522	1531	1540	1549	1558	1567
CAG GTC TCT AGG GCT ATA GGC GTG CGG GCT GGG CTG CTC AGC AGC CCA GTG GCC					
Q V S R A I G V R A G L L S S P V A					
1576	1585	1594	1603	1612	1621
CGT AGT GGG CAG GAG CCC CAG CCC AGT GGC AGC TGC ACT CGC TCA CAC CGT CGT					
R S G Q E P Q P S G S C T R S H R R					
1630	1639	1648	1657	1666	1675
CTG TCT GTC CAC CAC CTG GTC CAC CAC CAT CAC CAC CAT CAC TAC CAC					
L S V H H L V H H H H H H H H H H Y H					
1684	1693	1702	1711	1720	1729
CTG GGT AAT GGG ACG CTC AGA GTT CCC CGG GCC AGC CCA GAG ATC CAG GAC AGG					
L G N G T L R V P R A S P E I Q D R					
1738	1747	1756	1765	1774	1783
GAT GCC AAT GGG TCT CGC CGG CTC ATG CTA CCA CCA CCC TCT ACA CCC ACT CCC					
D A N G S R R L M L P P P S T P T P					
1792	1801	1810	1819	1828	1837
TCT GGG GGC CCT CCG AGG GGT GCG GAG TCT GTA CAC AGC TTC TAC CAT GCT GAC					
S G G P P R G A E S V H S F Y H A D					
1846	1855	1864	1873	1882	1891
TGC CAC TTG GAG CCA GTC CGT TGC CAG GCA CCC CCT CCC AGA TGC CCA TCG GAG					
C H L E P V R C Q A P P P R C P S E					
1900	1909	1918	1927	1936	1945
GCA TCT GGT AGG ACT GTG GGT AGT GGG AAG GTG TAC CCC ACT GTG CAT ACC AGC					
A S G R T V G S G K V Y P T V H T S					
1954	1963	1972	1981	1990	1999
CCT CCA CCA GAG ATA CTG AAG GAT AAA GCA CTA GTG GAG GTG GCC CCC AGC CCT					
P P P E I L K D K A L V E V A P S P					
2008	2017	2026	2035	2044	2053
GGG CCC CCC ACC CTC ACC AGC TTC AAC ATC CCA CCT GGG CCC TIC AGC TCC ATG					
G P P T L T S F N I P P G P F S S M					

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DNASIS DNA Translation [T-INS]

2062	2071	2080	2089	2098	2107
CAC AAG CTC CTG GAG ACA CAG AGT ACG GGA GCC TGC CAT AGC TCC TGC AAA ATC					
H K L L E T Q S T G A C H S S C K I					
2116	2125	2134	2143	2152	2161
TCC AGC CCT TGC TCC AAG GCA GAC AGT GGA GCC TGC GGG CCG GAC AGT TGT CCC					
S S P C S K A D S G A C G P D S C P					
2170	2179	2188	2197	2206	2215
TAC TGT GCC CGG ACA GGA GCA GGA GAG CCA GAG TCC GCT GAC CRT GTC ATG CCT					
Y C A R T G A G E P E S A D H V M P					
2224	2233	2242	2251	2260	2269
GAC TCA GAC AGC GAG GCT GTG TAT GAG TTC ACA CAG GAC GCT CAG CAC AGT GAC					
D S D S E A V Y E F T Q D A Q H S D					
2278	2287	2296	2305	2314	2323
CTC CCG GAT CCC CAC AGC CGG CGG CGA CAG CGG AGC CTG GGC CCA GAT GCA GAG					
L R D P H S R R R Q R S L G P D A E					
2332	2341	2350	2359	2368	2377
CCT AGT TCT GTG CTG GCT TTC TGG AGG CTG ATC TGT GAC ACA TTC CGG AAG ATC					
P S S V L A F W R L I C D T F R K I					
2386	2395	2404	2413	2422	2431
GTA GAT AGC AAA TAC TTT GGC CGG GGA ATC ATG ATC GCC ATC CTG GTC AAT ACA					
V D S K Y F G R G I M T A I L V N T					
2440	2449	2458	2467	2476	2485
CTC AGC ATG GGC ATC GAG TAC CAC GAG CAG CCC GAG GAG CTC ACC AAC GCC CTG					
L S M G I E Y H E Q P E E L T N A L					
2494	2503	2512	2521	2530	2539
GAA ATC AGC AAC ATC GTC TTC ACC AGC CTC TTC GCC TTG GAG ATG CTG CTG AAA					
E I S N I V F T S L F A L E M L L K					
2548	2557	2566	2575	2584	2593
CTG CTT GTC TAC GGT CCC TTT GGC TAC ATT AAG AAT CCC TAC AAC ATC TTT GAT					
L L V Y G P F G Y I K N P Y N I F D					
2602	2611	2620	2629	2638	2647
GGT GTC ATT GTG GTC ATC AGT GTG TGG GAG ATT GTG GGC CAG CAG GGA CGT GGC					
G V I V V I S V W E I V G Q Q G G G					
2656	2665	2674	2683	2692	2701
CTG TCG GTG CTG CGG ACC TTC CGC CTG ATG CGG GTG CTG AAG CTG GTG CGC TTC					
L S V L R T F R L M R V L K L V R F					
2710	2719	2728	2737	2746	2755
CTG CGG GCC CTG CAG CGC CAG CTC GTG GTG CTC ATG AAG ACC ATG GAC AAC GTG					
L P A L Q R Q L V V L M K T M D N V					

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DNASIS DNA Translation [T-INS]

2764	2773	2782	2791	2800	2809
GCC ACC TTC TGC ATG CTC CTC ATG CTG TTC ATC TTC ATC AGC ATC CTG GGC					
A T F C M L L M L F I F I F S I L G					
2818	2827	2836	2845	2854	2863
ATG CAT CTC TTT GGT TGC AAG TTC GCA TCT GAA CGG GAT GGG GAC ACG TTG CCA					
M H L F G C K F A S E R D G D T L P					
2872	2881	2890	2899	2908	2917
GAC CGG AAG AAT TTC GAC TCC CTG CTC TGG GCC ATC GTC ACT GTC TTT CAG ATT					
D R K N F D S L L W A I V T V F Q I					
2926	2935	2944	2953	2962	2971
CIG ACT CAG GAA GAC TGG AAT AAA GTC CTC TAC AAC GGC ATG GCC TCC ACA TCG					
L T Q E D W N K V L Y N G M A S T S					
2980	2989	2998	3007	3016	3025
TCT TGG GCT CTT TAC TTC ATC GCC CTC ATG ACT TTT GGC AAC TAT GTG CTC					
S W A A L Y F I A L M T F G N Y V L					
3034	3043	3052	3061	3070	3079
TTT AAC CTG CTG GTG GCC ATT CTT GTG GAA GGA TTC CAG GCA GAG GAA ATC GGC					
F N L L V A I L V E G F Q A E E I G					
3088	3097	3106	3115	3124	3133
AAA CGG GAA GAT GCG AGT GGA CAG TTA AGC TGT ATT CAG CTG CCT GTC AAC TCT					
K R E D A S G Q L S C I Q L P V N S					
3142	3151	3160	3169	3178	3187
CAG GGG GGA GAT GCC ACC AAG TCT GAG TCA GAG CCT GAT TTC TTT TCG CCC AGT					
Q G G D A T K S E S E P D F F S P S					
3196	3205	3214	3223	3232	3241
GTG GAT GGT GAT GGG GAC AGA AAG AAG CGC TTG GCC CTG GTG GCT TTG GGA GAA					
V D G D G D R K K R L A L V A L G E					
3250	3259	3268	3277	3285	3295
CAC GCG GAA CTA CGA AAG AGC CTT TTG CCA CCC CTC ATC ATC CAT ACG GCT GCG					
H A E L R K S L L P P L I I H T A A					
3304	3313	3322	3331	3340	3349
ACA CCA ATG TCA CTA CCC AAG AGC TCC AGC ACA GGT GTG GGG GAA GCA CTG GGC					
T P M S L P K S S S T G V G E A L G					
3358	3367	3376	3385	3394	3403
TCT CGC TCT CGA CGT ACC AGT AGC AGT GGG TCC GGT GAG CCT GGA GCT GCC CAC					
S G S R R T S S S G S A E P G A A H					
3412	3421	3430	3439	3448	3457
CAT GAG ATG AAA TCT CCG CCA AGT GGC CGC AGC TCC CCG CAC AGT CCC TGG AGT					
H E M K S P P S A R S S P H S P W S					

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DNASIS DNA Translation [T-INS]

3466	3475	3484	3493	3502	3511
GCG GCA AGC AGC TGG ACC AGC AGG CGC TCC AGC AGG AAC AGC CTG GGC CGG GCC					
---	---	---	---	---	---
A A S S W T S R R S S R N S L G R A					
3520	3529	3538	3547	3556	3565
CCC AGC CTA AAG CGG AGG AGC CCG AGC GGG GAG CGG AGG TCC CTG CTG TCT GGA					
---	---	---	---	---	---
P S L K R R S P S G E R R S L L S G					
3574	3583	3592	3601	3610	3619
GAG GGC CAG GAG AGT CAG GAT GAG GAA AGT TCA GAA GAG GAC CGG GCC AGC					
---	---	---	---	---	---
E G Q E S Q D E E S S S E E D R A S					
3628	3637	3646	3655	3664	3673
CCA GCA GGC AGT GAC CAT CGC CAC AGG GGT TCC TTG GAA CGT GAG CCC AAG AGT					
---	---	---	---	---	---
P A G S D H R H R G S L E R E A K S					
3682	3691	3700	3709	3718	3727
TCC TTT GAC CTG CCT GAC ACT CTG CAG GTG CCG GGG CTG CAC CGC ACA GCC AGC					
---	---	---	---	---	---
S F D L P D T L Q V P G L H R T A S					
3736	3745	3754	3763	3772	3781
GGC CGG AGC TCT GCC TCT GAG CAC CAA GAC TGT ATT GGC AAG TCG GCT TCA GGG					
---	---	---	---	---	---
G R S S A S E H Q D C N G X S A S G					
3790	3799	3808	3817	3826	3835
CGT TTG GCC CGC ACC CTG AGG ACT GAT GAC CCC CAA CTG GAT GGG GAT GAT GAC					
---	---	---	---	---	---
R L A R T L R T D D P Q L D G D D D					
3844	3853	3862	3871	3880	3889
AAT GAT GAG GGA AAT CTG AGC AAA GGG GAA CGC ATA CAA GCC TGG GTC AGA TCC					
---	---	---	---	---	---
N D E G N L S K G E R I Q A W V R S					
3898	3907	3916	3925	3934	3943
CGG CCT CCT GCC TGT TGC CGA GAG CGA GAT TCC TGG TCG GCC TAT ATC TTT CCT					
---	---	---	---	---	---
R L P A C C R E R D S W S A Y I F P					
3952	3961	3970	3979	3988	3997
CCT CAG TCA AGG TTT CGT CTC CTG TGT CAC CGG ATC ATC ACC CAC AAG ATG TTT					
---	---	---	---	---	---
P Q S R F R L L C H R I I T H K M F					
4006	4015	4024	4033	4042	4051
GAC CAT GTG GTC CTC GTC ATC ATC TTC CTC AAC TGT ATC ACC ATC GCT ATG GAG					
---	---	---	---	---	---
D H V V L V I I F L N C I T I A M E					
4060	4069	4078	4087	4096	4105
GGC CCC AAA ATT GAC CCC CAC AGC GCT GAG CGC ATC TTC CTG ACC CTC TCC AAC					
---	---	---	---	---	---
R P K I D P H S A E R I F L T L S N					
4114	4123	4132	4141	4150	4159
TAC ATC TTC ACG GCA GTC TTT CTA GCT GAA ATG ACA GTG AAG GTG GTG GCA CTG					
---	---	---	---	---	---
Y I F T A V F L A E M T V K V V A L					

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DNASIS DNA Translation [T-INS]

4168	4177	4186	4195	4204	4213
GGC TGG TGC TTT GGG GAG CAG GCC TAC CTG CGC AGC AGC TGG AAT GTG CTG GAC					
G W C F G E Q A Y L R S S W N V L D					
4222	4231	4240	4249	4258	4267
GGC TTG CTG GTG CTC ATC TCC GTC ATC GAC ATC CTG GTC TCC ATG GTC TCC GAC					
G L L V L I S V I D I L V S M V S D					
4276	4285	4294	4303	4312	4321
AGC GGC ACC AAG ATC CTT GGC ATG CTG AGG GTG CTG CGG CTG CTG CGG ACC CTG					
S G T K I L G M L R V L R L L R T L					
4330	4339	4348	4357	4366	4375
CGT CCA CTC AGG GTC ATC AGC CGG GCC CAG GGA CTG AAG CTG GTG GTA GAG ACT					
R P L R V I S R A Q G L K L V V E T					
4384	4393	4402	4411	4420	4429
CTG ATG TCA TCC CTC AAA CCC ATT GGC AAC ATT GTG GTC ATT TGC TGT GCC TTC					
L M S S L K P I G N I V V I C C A F					
4438	4447	4456	4465	4474	4483
TTC ATC ATT TTT GGA ATT CTC GGG GTG CAG CTC TTC AAA GGG AAG TTC TTC GTG					
F I I F G I L G V Q L F K G K F F V					
4492	4501	4510	4519	4528	4537
TGT CAG GGT GAG GAC ACC AGG AAC ATC ACT AAC AAA TCC GAC TGC GCT GAG GCC					
C Q G E D T R N I T N K S D C A E A					
4546	4555	4564	4573	4582	4591
AGC TAC CGA TGG GTC CGG CAC AAG TAC AAC TTT GAC AAC CTG GGC CAG GCT CTG					
S Y R W V R H K Y N F D N L G Q A L					
4600	4609	4618	4627	4636	4645
ATG TCC CTG TTT GTG CTG GCC TCC AAG GAT GGT TGG GTT GAC ATC ATG TAT GAT					
M S L F V L A S K D G W V D I M Y D					
4654	4663	4672	4681	4690	4699
GGG CTG GAT GCT GTG GGT GTG GAT CAG CAG CCC ATC ATG AAC CAC AAC CCC TGG					
G L D A V G V D Q Q P I M N H N P W					
4708	4717	4726	4735	4744	4753
ATG CTG CTA TAC TTC ATC TCC TTC CTC ATC GTG GCC TTC TTT GTC CTG AAC					
M L L Y F I S F L L I V A F F V L N					
4762	4771	4780	4789	4798	4807
ATG TTT GTG GGC GTG GTG GAG AAC TTC CAT AAG TGC AGA CAG CAC CAG GAG					
M F V G V V V E N F H K C R Q H Q E					
4816	4825	4834	4843	4852	4861
GAG GAG GAG GCG AGG CGG CGT GAG GAG AAG CGA CTA CGG AGG CTG GAG AAA AAG					
E E E A R R R E E K R L R R L E K K					

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DNASIS DNA Translation [T-INS]

4870	4879	4888	4897	4906	4915
AGA AGG AAT CTA ATG TTG GAC GAT GTA ATT GCT TCC GGC ACC TCA GCC AGC GCT					
R R N L M L D D V I A S G S S A S A					
4924	4933	4942	4951	4960	4969
GCG TCA GAA GCC CAG TGC AAG CCC TAC TAC TCT GAC TAC TCG AGA TTC CGG CTC					
A S E A Q C K P Y Y S D Y S R F R L					
4978	4987	4996	5005	5014	5023
CTT GTC CAC CAC CTG TGT ACC AGC CAC TAC CTG GAC CTC TTC ATC ACT GGT GTC					
L V H H L C T S H Y L D L F I T G V					
5032	5041	5050	5059	5068	5077
ATC GGG CTG AAC GTG GTC ACT ATG GCC ATG GAA CAT TAC CAG CAG CCC CGG ATC					
I G L N V V T M A M E H Y Q Q P Q I					
5086	5095	5104	5113	5122	5131
CTG GAC GAG GCT CTG AAG ATC TGC AAT TAC ATC TTT ACC GTC ATC TTT GTC TTT					
L D E A L K I C N Y I F T V I F V F					
5140	5149	5158	5167	5176	5185
GAG TCA GTT TTC AAA CTT GTG GCC TTT GGC TTC CGC CGT TTC CAG GAC AGG					
E S V F K L V A F G F R R F F Q D R					
5194	5203	5212	5221	5230	5239
TGG AAC CAG CTG GAC CTG GCT ATT GTG CTT CTG TCC ATC ATG GGC ATC ACA CTG					
W N Q L D L A I V L L S I M G I T L					
5248	5257	5266	5275	5284	5293
GAG GAG ATT GAG GTC AAT GCT TCG CTG CCC ATC AAC CCC ACC ATC ATC CGT ATC					
E E I E V N A S L P I N P T I I R I					
5302	5311	5320	5329	5338	5347
ATG AGG GTG CTC CGC ATT GCT CGA GTT CTG AAG CTG TTG AAG ATG GCT GTG GGC					
M R V L R I A R V L K L L K M A V G					
5356	5365	5374	5383	5392	5401
ATG CGG GCA CTG CTG GAC ACG GTG ATG CAG GGC CTG CCC CAG GTG GGG AAC CTG					
M R A L L D T V M Q A L P Q V G N L					
5410	5419	5428	5437	5446	5455
GGA CTT CTC TTC ATG TTA TTG TTT TTC ATC TTT GCA GCT CTG GGC GTG GAG CTC					
G L L F M L L F F I F A A L G V E L					
5464	5473	5482	5491	5500	5509
TTT GGA GAC CTG GAG TGT GAT GAG ACA CAC CCT TGT GAG GGC TTG GGT CGG CAT					
F G D L E C D E T H P C E G L G R H					
5518	5527	5536	5545	5554	5563
GCC ACC TTT AGG AAC TTT GGT ATG GCC TTT CTG ACC CTC TTC CGA GTC TCC ACT					
A T F R N F G M A F L T L F R V S T					

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DNASIS DNA Translation [T-INS]

5572	5581	5590	5599	5608	5617
GGT GAC AAC TGG AAT GGT ATT ATG AAG GAC ACC CTC CGG GAC TGT GAC CAG GAG					
G D N W N G I M K D T L R D C D Q E					
5626	5635	5644	5653	5662	5671
TCC ACC TGC TAC AAC ACT GTC ATC TCC CCT ATC TAC TTT GTG TCC TTC GTG CTG					
S T C Y N T V I S P I Y F V S F V L					
5680	5689	5698	5707	5716	5725
ACG GCC CAG TTT GTG CTG GTC AAC GTG GTC ATA GCT GTG CTG ATG AAG CAC CTG					
T A Q F V L V N V V I A V L M K H L					
5734	5743	5752	5761	5770	5779
GAA GAA AGC AAC AAA GAG GCC AAG GAG GAG GCC GAG CTC GAG GCC GAG CTG GAG					
E E S N K E A K E E A E L E A E L E					
5788	5797	5806	5815	5824	5833
CTG GAG ATG AAG ACG CTC AGC CCG CAG CCC CAC TCC CCG CTG GGC AGC CCC TTC					
L E M K T L S P Q P H S P L G S P F					
5842	5851	5860	5869	5878	5887
CTC TGG CCC CGG GTG GAG GGT GTC AAC AGT CCT GAC AGC CCT AAG CCT GGG GCT					
L W P G V E G V N S P D S P K P G A					
5896	5905	5914	5923	5932	5941
CCA CAC ACC ACT GCC CAC ATT GGA GCA GCC TCG GGC TTC TCC CTT GAG CAC CCC					
P H T T A H I G A A S G F S L E H P					
5950	5959	5968	5977	5986	5995
ACG ATG GTA CCC CAC CCC GAG GAG GTG CCA GTC CCC CTA GGA CCA GAC CTG CTG					
T M V P H P E E V P V P L G P D L L					
6004	6013	6022	6031	6040	6049
ACT GTG AGG AAG TCT GGT GTC AGC CGG ACG CAC TCT CTG CCC AAT GAC AGC TAC					
T V R K S G V S R T H S L P N D S Y					
6058	6067	6076	6085	6094	6103
ATG TGC CGC AAT GGG AGC ACT GCT GAG AGA TCC CTA GGA CAC AGG GGC TGG GGG					
M C R N G S T A E R S L G H R G W G					
6112	6121	6130	6139	6148	6157
CTC CCC AAA GCC CAG TCA GGC TCC ATC TTG TCC GTT CAC TCC CAA CCA GCA GAC					
L P K A Q S G S I L S V H S Q P A D					
6166	6175	6184	6193	6202	6211
ACC AGC TGC ATC CTA CAG CTT CCC AAA GAT GTG CAC TAT CTG CTC CAG CCT CAT					
T S C I L Q L P K D V H Y L L Q P H					
6220	6229	6238	6247	6256	6265
GGG GCC CCC ACC TGG GGC GCC ATC CCT AAA CTA CCC CCA CCT GGC CGC TCC CCT					
G A P T W G A I P K L P P P G R S P					

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DNASIS DNA Translation [T-INS]

6274	6283	6292	6301	6310	6319
CTG GCT CAG AGG CCT CTC AGG CGC CAG GCA GCA ATA AGG ACT GAC TCC CTG GAT					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
L A Q R P L R R Q A A I R T D S L D					
6328	6337	6346	6355	6364	6373
G TG CAG GGC CTG GGT AGC CGG GAA GAC CTG TTG TCA GAG GTG AGT GGG CCC TCC					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
V Q G L G S R E D L L S E V S G P S					
6382	6391	6400	6409	6418	6427
TGC CCT CTG ACC CGG TCC TCA TCC TTC TGG GGC GGG TCG AGC ATC CAG GTG CGG					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
C P L T R S S S F W G G S S I Q V Q					
6436	6445	6454	6463	6472	6481
CAG CGT TCC GGC ATC CAG AGC AAA GTC TCC AAG CAC ATC CGC CTG CCA GCC CCT					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
Q R S G I Q S K V S K H I R L P A P					
6490	6499	6508	6517	6526	6535
TGC CCA GGC CTG GAA CCC AGC TGG GCC AAG GAC CCT CCA GAG ACC AGA AGC AGC					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
C P G L E P S W A K D P P E T R S S					
6544	6553	6562	6571	6580	6589
TTA GAG CTG GAC AGC GAG CTG AGC TGG ATT TCA GGA GAC CTC CTT CCC AGC AGC					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
L E L D T E L S W I S G D L L P S S					
6598	6607	6616	6625	6634	6643
CAG GAA GAA CCC CTG TCC CCA CGG GAC CTG AAG AAG TGC TAC AGT GTA GAG ACC					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
Q E E P L S P R D L K K C Y S V E T					
6652	6661	6670	6679	6688	6697
CAG AGC TGC AGG CGC AGG CCT GGG TCC TGG CTA GAT GAA CAG CGG AGA CAC TCC					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
Q S C R R R P G S W L D E Q R R H S					
6706	6715	6724	6733	6742	6751
ATT GCT GTC AGC TGT CTG GAC AGC GGC TCC CAA CCC CGC CTA TGT CCA AGC CCC					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
I A V S C L D S G S Q P R L C P S P					
6760	6769	6778	6787	6796	6805
TCA AGC CTC GGG GGC CAA CCT CTT GGG GGT CCT GGG AGC CGG CCT AAG AAA AAA					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
S S L G G Q P L G G P G S R P K K K					
6814	6823	6832	6841	6850	6859
CTC AGC CCA CCC AGT ATC TCT ATA GAC CCC CCG GAG AGC CAG GGC TCT CGG CCC					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
L S P P S I S I D P P E S Q G S R P					
6868	6877	6886	6895	6904	6913
CCA TGC AGT CCT GGT GTC TGC CTC AGG AGG AGG CGG CGG GCC AGT GAC TCT AAG					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
P C S P G V C L R R R A P A S D S K					
6922	6931	6940	6949	6958	6967
GAT CCC TCG GTC TCC AGC CCC CTT GAC AGC AGC GCT GCC TCA CCC TCC CCA AAG					
- - - - -	- - - - -	- - - - -	- - - - -	- - - - -	- - - - -
D P S V S S P L D S T A A S P S P K					

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DNASIS DNA Translation [T-INS]

6976	6985	6994	7003	7012	7021
AAA GAC ACG CTG AGT CTC TCT GGT TTG TCT TCT GAC CCA ACA GAC ATG GAC CCC					
K D T L S L S G L S S D P T D M D P					
7030	7039	7048	7057	7066	7075
TGA GTC CTA CCC ACT CTC CCC CAT CAC CTT TCT CCA CCG GGT GCA GAT CCT AGC					
* V L P T L P H H L S P P G A D P S					
7084	7093	7102	7111	7120	7129
TCC GCC TCC TGG GCA GCG TTT CTG AAA AGT CCC ACG TAA GCA GCA AGC AGC CAC					
S A S W A A F L K S P T * A A S S H					
7138	7147	7156	7165	7174	7183
GAG GCA CCT CAC CTG CCT TCT TCA GTG GCT GGT GGG GAT GAC GAG CAG AAC TTC					
E A P H L P S S V A G G D D E Q N F					
7192	7201	7210	7219	7228	7237
CGG AGA GTC GAT CTG AAG AGA ACA CAG CCC TGG AGC CCC TGC CTC CGG GAA GAA					
R R V D L K R T Q P W S P C L R E E					
7246	7255	7264	7273	7282	56Q 1
GGA AAA GGA GAA AGC CCA GTG TGG CCA AGG CTC CCG ACA CCA GGA GCT G/3'					
G K G E S P V W P R L P T P G A / 56Q 2 4					

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5' → 3' No: 3

10	20	30	40	50	60
GEAGCTGAGC	TGAACCTGGCC	CTCCCTGGGGA	CTCAGCAAGC	TCTCTAGAGC	CCCCCACATG
70	80	90	100	110	120
CTCCCCCACC	GGGGTCCCCC	GGTTGCGTGA	GGACACCTCC	TCTGAGGGGC	TCGGCTGCC
130	140	150	160	170	180
CCTCTTCGGA	CCCCCCCAGGG	CCCCGGCTGG	CCAGAGGATG	GACGGAGGAGG	AGGATGGAGC
190	200	210	220	230	240
GGGGCGCGAG	GAGTCGGGAC	AGCCCCGTAG	CTTCACGCAG	CTCAACGACC	TGTCCGGGC
250	260	270	280	290	300
CGGGGGCCGG	CAGGGGCCGG	GGTCGACCGA	AAAGGACCCG	GGCAAGCGCG	ACTCCGAGGC
310	320	330	340	350	360
GGAGGGGCTG	CCGTACCCGG	CCCTAGCCCC	GGTGGTTTTC	TTCTACTTGA	GGCAGGACAG
370	380	390	400	410	420
CCGGCCCGCGG	AGCTGGGTGTC	TCCGCACGGT	CTGTAACCCG	TGGTTCGAGC	GAGTCAGTAT
430	440	450	460	470	480
GCTGGTCATT	CTTCTCAACT	GTGTGACTCT	GGGTATGTT	AGGCCGTGTG	AGGACATTGC
490	500	510	520	530	540
CTGTGACTCC	CAGCGCTGCC	GGATCCTGCA	GGCCTTCGAT	GACTTCATCT	TTGCCCTTC
550	560	570	580	590	600
TGCTGTGGAA	ATGGTGGTGA	AGATGGGAGC	CTTGGGCATC	TTTGGGAAGA	AATGTTACCT
610	620	630	640	650	660
GGGAGACACT	TGGAACCGGC	TTGACTTTT	CATTGTCATT	CCAGGGATGC	TGGAGTATT
670	680	690	700	710	720
GCTGGACCTG	CAGAACGTCA	CCCTCTCCGC	AGTCAGGACA	GTCCGTGTG	TGGCACCGCT
730	740	750	760	770	780
CAGGGCCATT	AACCGGGTGC	CCAGCATGCG	CATTCTCGTC	ACATTACTGC	TGGACACCTT
790	800	810	820	830	840
GCCTATGCTG	GGCAACGTCC	TGCTGCTCTG	TTTCTTCGTC	TTTTTCATCT	TGGGCATCGT
850	860	870	880	890	900
GGCGCGTCCAG	CTGTGGGAG	GAETGCTTCG	CAACCGATGC	TTCCCTCCCCG	AGAACCTTCAG
910	920	930	940	950	960
CCTCCCCCTG	ACCGTGGAC	TGGAGCCTA	TTACCAAGACA	GAGAATGAGG	ACGGAGAGCCC
970	980	990	1000	1010	1020
CTICATCTGC	TCTCAGCCCTC	GGGAGAATGG	CATGAGATCC	TGGCAGGAGTG	TGCCCAACT
1030	1040	1050	1060	1070	1080
GGGTGGGAA	GGCGGTGGTG	CCCCACCCCTG	CAGTCCTGGAC	TATGAGACCT	ATAACAGTT
1090	1100	1110	1120	1130	1140
CAGCAACACC	ACCTGTGTCA	ACTGGAACCA	GTACTATACC	AACTGCTCTG	CGGGCGAGCA
1150	1160	1170	1180	1190	1200
CAACCCCTTC	AAAGGCGCCA	TCAACTTGA	CAACATIGGC	TATGCCCTGGA	TCGCCATCTT
1210	1220	1230	1240	1250	1260
CCAGGTCACT	ACACTGGAGG	GCTGGGTGGA	CATCATGTAC	TTCGTAATGG	ACGGCTGACTC

Start of
SEQ ID NO: 1
coding region

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DNASIS
T-INS

1270	1280	1290	1300	1310	1320
CTTCTACAAAC	TTCATCTACT	TCATCTCTCT	CATCATCGTG	GGCTCCCTCT	TCATGATCAA
1330	1340	1350	1360	1370	1380
CCTGTGCCCTG	GTTGGTATTG	CCACCGAGTT	CTCCCGAGACC	AAACAGCGGG	AGAGTCAGCT
1390	1400	1410	1420	1430	1440
GATGCGGGGAG	CAGCGTGTAC	GATTCCCTGTC	CAATGCTAGC	ACCCCTGGAA	GCTTCTCTGA
1450	1460	1470	1480	1490	1500
GCCAGGCAGC	TGCTATGAGG	AGCTACTICAA	GTACCTGGTG	TACATCCCTC	AAAAAGCAGC
1510	1520	1530	1540	1550	1560
CCGAAGGCCTG	GCCCCAGGTCT	CTAGGGCTAT	AGGGCTGCGG	GCTGGGCTGC	TCAGCAGCCC
1570	1580	1590	1600	1610	1620
AGTGGCCCGT	AGTGGGCAGG	AGCCGCCAGCC	CAGTGGCAGC	TGCACTCGCT	CACACCGTCTG
1630	1640	1650	1660	1670	1680
TCTGTCTGTC	CACCACTCTGG	TCCACCACCA	TCACCAACAC	CATCACCACT	ACCACTGGGG
1690	1700	1710	1720	1730	1740
TAATGGGACG	CTCAGAGTTC	CCCGGGCCAG	CCCAGAGATC	CAGGACAGGG	ATGCCAATGG
1750	1760	1770	1780	1790	1800
GTCCTGCCGG	CTCATGCTAC	CACCACTCTC	TACACCCACT	CCCTCTGGGG	GCCCTCCGAG
1810	1820	1830	1840	1850	1860
GGGTGCGGAG	TCTGTACACA	GCTTCTACCA	TGCTGACTGC	CACTTGGAGC	CAGTCCTGTTG
1870	1880	1890	1900	1910	1920
CCAGGCACCC	CCTCCCCAGAT	GCCCCATCGGA	GGCATCTGGT	AGGACTGTGG	GTAGTGGGAA
1930	1940	1950	1960	1970	1980
GGTGTACCCC	ACTGTGCAATA	CCAGCCCTCC	ACCAGAGATA	CTGAAGGATA	AAGCACTAGT
1990	2000	2010	2020	2030	2040
GGAGGTGGCC	CCCAGCCCTG	GGCCCCCCCAC	CCTCACCAAGC	TTCAACATCC	CACCTGGGCC
2050	2060	2070	2080	2090	2100
CTTCAGCTCC	ATGCACAAGC	TCCTGGAGAC	ACAGACTACG	GGAGCTGCG	ATAGCTCTG
2110	2120	2130	2140	2150	2160
CAAAATCTCC	AGCCCTTGCT	CCAAGGCAGA	CAGTGGAGCC	TGCGGGCCGG	ACAGTTGTCC
2170	2180	2190	2200	2210	2220
CTACTGTGCC	CGGACAGGAG	CAGGAGAGCC	AGAGTCCGCT	GACCATGTC	TGCTCTGACTC
2230	2240	2250	2260	2270	2280
AGACACCGAG	GCTGTGTAATG	AGTTCACACA	GGACGCTCAG	CACACTGAC	TCCGGGATCC
2290	2300	2310	2320	2330	2340
CCACAGCCGG	CGGCGACAGC	GGAGCTGGG	CCCAGATGCA	GAGCCTAGTT	CTGTGCTGGC
2350	2360	2370	2380	2390	2400
TTTCTGGAGG	CTGATCTGTC	ACACATTCCG	GAAGATCGTA	GATAGCAAAT	ACTTIGCCCG
2410	2420	2430	2440	2450	2460
GGGAATCATG	ATCGCCATCC	TGGTCAATAC	ACTCAGGATG	GGCATCGAGT	ACCACGAGCA
2470	2480	2490	2500	2510	2520
CCCCGAGGAG	CTCACCAACG	CCCTGGAAAT	CAGCAACATC	GTCTTCACCA	GCCTCTTOGC

2530 2540 2550 2560 2570 2580
 CTGGAGATG CTGGTGAAC TGCTTGCTA CGGTCCCTT GGCTACATTA AGAATCCCTA
 2590 2600 2610 2620 2630 2640
 CAACATCTT GATGGTGTCA TTGTGGTCAT CAGTGTGTGG GAGATTGTGG CCCAGCAGGG
 2650 2660 2670 2680 2690 2700
 AGGTGGCTG TCGGTGCTGC GGACCTTCGG CCTGATGCCG GTGCTGAAGC TGGTGGCTT
 2710 2720 2730 2740 2750 2760
 CCTGCCGCCCT CTGCAGGCC AGCTCGGGT GCTCATGAAG ACCATGGACA ACGTGGCCAC
 2770 2780 2790 2800 2810 2820
 CTCTGCAATG CTCCATCATGC TGTTCATCTT CNTCTTCAGC ATCCTGGCA TGCATCTCTT
 2830 2840 2850 2860 2870 2880
 TGGTTGCAAG TTGGCATCTG AACGGGATGG CGACACGTTG CCAGACCGGA AGAATTTCGA
 2890 2900 2910 2920 2930 2940
 CTCCCCTGCTC TGGCCCATCG TCACTGTCTT TCAGATTCTG ACTCAGGAG ACTGGAATAA
 2950 2960 2970 2980 2990 3000
 AGTCCCTCTAC AACGGCATGG CCTCCACATC GTCTGGGCT GCTCTTACT TCATCGCCCT
 3010 3020 3030 3040 3050 3060
 CATGACTTTT GGCAACTATG TGCTCTTAA CCTGCTGGTG GCCATTCTTG TGGAGGATT
 3070 3080 3090 3100 3110 3120
 CCAGGCAGAG GAAATCGGCA AACGGGAAGA TGGGAGTGGGAG CAGTTAACCT GTATTCAAGCT
 3130 3140 3150 3160 3170 3180
 GCCTGTCAAC TCTCAGGGGG GAGATGCCAC CAAGTCTGAG TCAGAGCCTG ATTTCTTTC
 3190 3200 3210 3220 3230 3240
 GCCCAGTGTG GATGGTGTAG GGGACAGAAA GAAGGGCTTG GCCCTGGTGG CTTTGGGAGA
 3250 3260 3270 3280 3290 3300
 ACACCCGGAA CTACGAAAGA GCCTTTTGCC ACCCCTCATC ATCCATACGG CTGCGACACC
 3310 3320 3330 3340 3350 3360
 AATGTCACTA CCCAAGAGCT CCAGCACAGG TGTGGGGAA GCACGGGCT CTGGCTCTCG
 3370 3380 3390 3400 3410 3420
 ACGTACCACT AGCACTGGGT CCGCTGAGCC TGGAGCTGCC CACCATGAGA TGAATCTCC
 3430 3440 3450 3460 3470 3480
 GCCAAGTGCC CGCAGCTCCC CGCACAGTCC CTGGAGTGGC GCAAGCAGCT GGACCACAG
 3490 3500 3510 3520 3530 3540
 GCGCTCCAGC AGGAACAGCC TGGGCCGGC CCCCCAGCCTA AAGCGGAGGA GCGCGACCGG
 3550 3560 3570 3580 3590 3600
 GGAGCCGGAGG TCCCTGCTGT CTGGAGAGGG CCAGGAGAGT CAGGATGAGG AGGAAAGTTC
 3610 3620 3630 3640 3650 3660
 AGAAGAGGAC CGGGCCAGCC CAGCACAGG TGACCATCGC CACAGGGGTT CCTTGGAACG
 3670 3680 3690 3700 3710 3720
 TGAGGCCAAG AGTTCCTTIG ACCTGCCCTGA CACTCTGCAG GTGCCGGGC TGCACCGCAC
 3730 3740 3750 3760 3770 3780
 AGCCAGCCGC CGGAGCTCTG CCTCTGAGCA CCAAGACTGT AATGCCAAGT CGGCTTCAGG

3790 3800 3810 3820 3830 3840
 GCGTTTGGCC CGCACCCGAGA GGACTGATGA CCCCCAACTG GATGGGGATG ATGACAAATGA
 3850 3860 3870 3880 3890 3900
 TGAGGGAAAT CTGAGCAAAG GGGARCGCAT ACAAGCCTGG GTCAGATCCC GGCTTCCTGC
 3910 3920 3930 3940 3950 3960
 CTGTTGCCGA GAGGGAGATT CCTGGTCGGC CTATATCTTT CCTCCCTAGT CAAGGTTTCG
 3970 3980 3990 4000 4010 4020
 TCTCCCTGTGTCACCGGATCA TCACCCACAA GATGTTTGAC CATGTGGTCC TCGTCATCAT
 4030 4040 4050 4060 4070 4080
 CTTCCCTCAAC TGTATCACCA TCGCTATGGA GCGCCCCAAA ATTGACCCCC ACAGCGCTGA
 4090 4100 4110 4120 4130 4140
 GGGCATCTTC CTGACCCCTCT CCAACTACAT CTTACCGGCA GTCTTCTCTAG CTGAAATGAC
 4150 4160 4170 4180 4190 4200
 AGTGAAGGTG GTGGCACTGG GCTGGTGCTT TGGGGAGCAG GCCTACCTGC GCAGCAGCTG
 4210 4220 4230 4240 4250 4260
 GAATGTGCTG GACGGCTTGC TGGTGCTCAT CTCCGTCATC GACATCCCTGG TCTCCATGGT
 4270 4280 4290 4300 4310 4320
 CTCCGACAGC GGCACCAAGA TCCTTGGCAT GCTGAGGGTG CTGGGGCTGC TGCGGACCCCT
 4330 4340 4350 4360 4370 4380
 CGGTCCCACTC AGGGTCATCA GCGGGGCCA GGGACTGAAG CTGGTGGTAG AGACTCTGAT
 4390 4400 4410 4420 4430 4440
 GTCATCCCTC AAACCCATGT GCAACATGTG GGTCATTTGC TGTCCTTCT TCATCATTTT
 4450 4460 4470 4480 4490 4500
 TGGAAATTCTC GGGGTGCAGC TCTTCAAAGG GAAGTTCTTC GTGTGTCAGG GTGAGGACAC
 4510 4520 4530 4540 4550 4560
 CAGGAACATC ACTAACAAAT CCGACTGGC TGAGGCCAGC TACCGATGGG TCCGGCACAA
 4570 4580 4590 4600 4610 4620
 GTACAACCTTT GACAACCTGG GCCAGGCCTCT GATGTCCTTG TTGTGCTGG CCTCCAAGGA
 4630 4640 4650 4660 4670 4680
 TCGTTGGGT GACATCATGT ATGATGGCT GGATGCTGTG GGTGTGGATC AGCAGCCAT
 4690 4700 4710 4720 4730 4740
 CATGAACCAC AACCCCTGGA TGCTGCTATA CTTCATCTCC TTCCCTCTCA TCGTGGCCTT
 4750 4760 4770 4780 4790 4800
 CTTTGTCTG AACATGTTTG TGGCCGTGGT GGTGGAGAAC TTCCATAAGT GCAGACAGCA
 4810 4820 4830 4840 4850 4860
 CCAGGAGGAG GAGGAGGCCA GCGGGCGTGA GGAGAAGCGA CTACGGAGGC TGGAGAAAAA
 4870 4880 4890 4900 4910 4920
 GAGAAGGAAT CTAATGTTGG ACCATGTAAT TGCTTCCGGC AGCTCAGCCA GCGCTGCCTC
 4930 4940 4950 4960 4970 4980
 AGAAGCCCAAG TGCAAGCCCT ACTACTCTGA CTACTCGAGA TTCCGGCTCC TTGTCCACCA
 4990 5000 5010 5020 5030 5040
 CCTGTGTACCG AGCCACTACC TGGACCCCTT CATCACTGGT GTCACTGGGC TGAACGTGGT

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5050 5060 5070 5080 5090 5100
 CACTATGGCC ATGGAACATT ACCAGCAGCC CCAGATCTG GACGAGGCTC TGAAGATCTG
 5110 5120 5130 5140 5150 5160
 CAATTACATC TTIAACCGTCA TCTTTGTCTT TGAGTCAGTT TTCAAACTTG TGGCCTTTGG
 5170 5180 5190 5200 5210 5220
 CTTCCGCCGT TTCTTCAGG ACAGGTGGAA CCAGCTGGAC CTGGCTATIG TGCTTCTGTC
 5230 5240 5250 5260 5270 5280
 CATCATGGGC ATCACACTGG AGGAGATTGA GGTCAATGCT TCGCTGCCA TCAACCCCAC
 5290 5300 5310 5320 5330 5340
 CATCATCCGT ATCATGAGGG TGCTCCGCAT TGCTCGAGTT CTGAGCTGT TGAAGATGGC
 5350 5360 5370 5380 5390 5400
 TGTGGGCATG CGGGCACTGC TGGACACGGT GATGCAGGCC CTGGCCCCAGG TGGGAACCT
 5410 5420 5430 5440 5450 5460
 GGGACTTCTC TTCATGTAT TGTTCATCTT CTTCAGCTT CTGGGCGTGG AGCTCTTGG
 5470 5480 5490 5500 5510 5520
 AGACCTGGAG TGTGATGAGA CACACCCCTTG TGAGGGCTTG GGTCGGCATG CCACCTTTAG
 5530 5540 5550 5560 5570 5580
 GAACCTTGCTT ATGGCCTTTC TGACCCCTT CCGAGCTCTCC ACTGGTGACA ACTGGAAATGG
 5590 5600 5610 5620 5630 5640
 TATTATGAAG GACACCCCTCC GGGACTGTGA CCAGGAGTCC ACCTGCTACA ACACGTGCT
 5650 5660 5670 5680 5690 5700
 CTCCCCCTATC TACTTGTGT CCTTCGTGCT GACGGCCAG TTGTGCTGG TCAACGGTGG
 5710 5720 5730 5740 5750 5760
 CATAGCTGTG CTGATGAAGC ACCTGGAAGA AAGCAACAAA GAGGCCAAGG AGGAGGCCGA
 5770 5780 5790 5800 5810 5820
 GCTCGAGGCC GAGCTGGAGC TGGAGATGAA GACGCTCAGC CGCGAGCCCC ACTCCCCGCT
 5830 5840 5850 5860 5870 5880
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 5890 5900 5910 5920 5930 5940
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 5950 5960 5970 5980 5990 6000
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 6010 6020 6030 6040 6050 6060
 GAGGAAGTCT GGTCAGCC GGACGCACTC TCTGCCCAAT GACAGCTACA TGTGCCGCAA
 6070 6080 6090 6100 6110 6120
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 6130 6140 6150 6160 6170 6180
 AGGCTCCATC TTGTCCGTTC ACTCCCCAACC AGCAGACACC AGCTGCATCC TACAGCTTCC
 6190 6200 6210 6220 6230 6240
 CAAAGATGTG CACTATCTGC TCCAGCCTCA TGGGGCCCCC ACCTGGGGCG CCATCCCTAA
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CCTGCCTCT	TCAGTGGCTG	GTGGGGATGA	CGAGCAGAAC	TTCCGGAGAG	TGATCTGAA
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7270	7280	7290	7300	7310	7320
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end of
SEQ 1 + 3

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SEQ 19 NO:4
 start of
 SEQ 10 NO:2
 (coding region)

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 F F I F G I V G V Q L W A G L L R N R C F L P E N F S L P L S V D L E P Y Y Q T E N E D E S
 P F I C S Q P R E N G M R S C R S V P T L R G E G G G G P P C S L D Y E T Y N S S N T T
 C V N W N Q Y Y T N C S A G E H N P F K G A I N F D N I G Y A W I A I F Q V I T L E G W V
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 F G N Y V L F N L L V A I L V E G F Q A E E I G K R E D A S G Q L S C I Q L P V N S Q G G D
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 P P S A R S S P H S P W S A A S S W T S R R S S R N S L G R A P S L K R R S P S G E R R S
 L L S G E G Q E S Q D E E E S S E E D R A S P A G S D H R H R G S L E R E A K S S F D L P D
 T L Q V P G L H R T A S G R S S A S E H Q D C N G K S A S G R L A R T L R T D D P Q L D G
 D D D N D E G N L S K G E R I Q A W V R S R L P A C C R E R D S W S A Y I F P P Q S R F R
 L L C H R I I T H K M F D H V V L V I I F L N C I T I A M E R P K I D P H S A E R I F L T L S N
 Y I F T A V F L A E M T V K V V A L G W C F G E Q A Y L R S S W N V L D G L L V L I S V I
 D I L V S M V S D S G T K I L G M L R V L R L L R T L R P L R V I S R A Q G L K L V V E T L
 M S S L K P I G N I V V I C C A F F I I F G I L G V Q L F K G K F F V C Q G E D T R N I T N K
 S D C A E A S Y R W V R H K Y N F D N L G Q A L M S L F V L A S K D G W V D I M Y D G L
 D A V G V D Q Q P I M H N P W M L L Y F I S F L L I V A F F V L N M F V G V V V E N F H
 K C R Q H Q E E E E A R R E E K R L R R L E K K R R N L M L D D V I A S G S S A A S
 E A Q C K P Y Y S D Y S R F R L L V H H L C T S H Y L D L F I T G V I G L N V V T M A M E
 H Y Q Q P Q I L D E A L K I C N Y I F T V I F V F E S V F K L V A F G F R R F F Q D R W N Q
 L D L A I V L L S I M G I T L E E I E V N A S L P I N P T I I R I M R V L R I A R V L K L L K M
 A V G M R A L L D T V M Q A L P Q V G N L G L L F M L L F I F A A L G V E L F G D L E C
 D E T H P C E G L G R H A T F R N F G M A F L T L F R V S T G D N W N G I M K D T L R D C
 D Q E S T C Y N T V I S P I Y F V S F V L T A Q F V L V N V V I A V L M K H L E E S N K E A
 K E E A E L E A E L E M K T L S P Q P H S P L G S P F L W P G V E G V N S P D S P K P G
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 T H S L P N D S Y M C R N G S T A E R S L G H R G W G L P K A Q S G S I L S V H S Q P A D
 T S C I L Q L P K D V H Y L L Q P H G A P T W G A I P K L P P P G R S P L A Q R P L R R Q A
 A I R T D S L D V Q G L G S R E D L L S E V S G P S C P L T R S S S F W G G S S I Q V Q Q R
 S G I Q S K V S K H I R L P A P C P G L E P S W A K D P P E T R S S L E L D T E L S W I S G
 D L L P S S Q E E P L S P R D L K K C Y S V E T Q S C R R R P G S W L D E Q R R H S I A V
 S C L D S G S Q P R L C P S P S S L G G Q P L G G P G S R P K K K L S P P S I S I D P P E S Q
 G S R P P C S P G V C L R R A P A S D S K D P S V S S P L D S T A A S P S P K K D T L S L
 S G L S S D P T D M D P * V L P T L P H H L S P P G A D P S S A S W A A F L K S P T * A A S
 S H 2 0 7 1 E A P H L P S S V A G G D D E Q N F R R V D L K R T Q P W S P C L R E E G K G E
 S P V W P R L P T P G A /
 end of
 SEQ 2+4

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SEQ ID NO:5: SKEKQMA

SEQ ID NO:6: 5' TNGC(A/C/T)ATGGAG(C/A)GNCC(C/T)-3'

SEQ ID NO:7: 5' -CTT(C/G/T)CCCTTGAA(G/C)A(G/A)CTG)-3'

SEQ ID NO:8: 5' -CCGCTGTCGGAGACCATGGAGACC-3'

SEQ ID NO:9: 5' -AGCGGCCAAAATTGACCCCCACAG-3'

SEQ ID NO:10: 5' -GAAGATGCGAGTGGACAG-3'

SEQ ID NO:11: 5' - CTGTGGCGATGGTCACTG-3'

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What Is Claimed Is:

1 1. An isolated nucleic acid molecule encoding a
2 pancreatic T-type calcium channel.

1 2. The isolated nucleic acid molecule of claim 1
2 wherein said nucleic acid is deoxyribonucleic acid.

1 3. The isolated nucleic acid molecule of claim 2
2 wherein said deoxyribonucleic acid is cDNA.

1 4. The isolated nucleic acid molecule of claim 3
2 wherein said nucleic acid molecule has a nucleotide
3 sequence as shown in SEQ ID NO:1.

1 5. The isolated nucleic acid molecule of claim 1
2 wherein said nucleic acid molecule encodes an amino acid
3 sequence as shown in SEQ ID NO:2.

1 6. The isolated nucleic acid molecule of claim 1
2 wherein said nucleic acid is ribonucleic acid.

1 7. The isolated nucleic acid molecule of claim 6
2 wherein said ribonucleic acid is mRNA.

1 8. An antisense nucleic acid molecule complementary
2 to at least a portion of the mRNA of claim 7.

1 9. A cell comprising the antisense nucleic acid
2 molecule of claim 8.

1 10. An expression vector comprising the antisense
2 nucleic acid molecule of claim 8.

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1 11. The expression vector of claim 10 wherein the
2 expression vector is selected from the group consisting
3 of a plasmid and a virus.

1 12. A cell comprising the expression vector of
2 claim 10.

1 13. A method of decreasing expression of a
2 pancreatic T-type calcium channel in a host cell, said
3 method comprising introducing the antisense nucleic acid
4 molecule of claim 8 into the cell, wherein said antisense
5 nucleic acid molecule blocks translation of said mRNA so
6 as to decrease expression of said pancreatic T-type
7 calcium channel in said host cell.

1 14. A ribozyme having a recognition sequence
2 complementary to a portion of the mRNA of claim 7.

1 15. A cell comprising the ribozyme of claim 14.

1 16. An expression vector comprising the ribozyme of
2 claim 14.

1 17. The expression vector of claim 16 wherein the
2 expression vector is selected from the group consisting
3 of a plasmid and a virus.

1 18. A cell comprising the expression vector of
2 claim 16.

1 19. A method of decreasing expression of a
2 pancreatic T-type calcium channel in a host cell, said
3 method comprising introducing the ribozyme of claim 14
4 into the cell, wherein expression of said ribozyme in

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5 said cell results in decreased expression of said
6 pancreatic T-type calcium channel in said cell.

1 20. A cell comprising the nucleic acid molecule of
2 claim 1.

1 21. An expression vector comprising the nucleic
2 acid molecule of claim 1.

1 22. The expression vector of claim 21 wherein said
2 expression vector is selected from the group consisting
3 of a plasmid and a virus.

1 23. A cell comprising the expression vector of
2 claim 21.

1 24. A method of increasing expression of pancreatic
2 T-type calcium channel in a host cell, said method
3 comprising:

4 introducing the nucleic acid molecule of
5 claim 1 into the cell; and

6 allowing said cell to express said nucleic acid
7 molecule resulting in the production of pancreatic T-type
8 calcium channel in said cell.

1 25. A method of screening a substance for the
2 ability of the substance to modify T-type calcium channel
3 function, said method comprising:

4 introducing the nucleic acid molecule of claim 1
5 into a host cell;

6 expressing said pancreatic T-type calcium channel
7 encoded by said nucleic acid molecule in the host cell;
8 exposing the cell to a substance; and

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9 evaluating the exposed cell to determine if the
10 substance modifies the function of the T-type calcium
11 channel.

1 26. The method of claim 25 wherein said evaluation—
2 comprises monitoring the expression of T-type calcium
3 channel.

1 27. A method of obtaining DNA encoding a pancreatic
2 T-type calcium channel, said method comprising:

3 selecting a DNA molecule encoding a pancreatic T-
4 type calcium channel, said DNA molecule having a
5 nucleotide sequence as shown in SEQ ID NO:1;

6 designing an oligonucleotide probe for a pancreatic
7 T-type calcium channel based on SEQ ID NO:1;

8 probing a genomic or cDNA library of an organism
9 with the oligonucleotide probe; and

10 obtaining clones from said library that are
11 recognized by said oligonucleotide probe, so as to obtain
12 DNA encoding a pancreatic T-type calcium channel.

1 28. A method of obtaining DNA encoding a pancreatic
2 T-type calcium channel, said method comprising:

3 selecting a DNA molecule encoding a pancreatic
4 T-type calcium channel, said DNA molecule having a
5 nucleotide sequence as shown in SEQ ID NO:1;

6 designing degenerate oligonucleotide primers
7 based on SEQ ID NO:1; and

8 utilizing said oligonucleotide primers in a
9 polymerase chain reaction on a DNA sample to identify
10 homologous DNA encoding a pancreatic T-type calcium
11 channel in said sample.

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1 29. An isolated nucleic acid molecule encoding a
2 pancreatic T-type calcium channel, said nucleic acid
3 molecule encoding a first amino acid sequence having at
4 least 90% amino acid identity to a second amino acid
5 sequence, said second amino acid sequence as shown in SEQ-
6 ID NO:2.

1 30. A DNA oligomer capable of hybridizing to the
2 nucleic acid molecule of claim 1.

1 31. A method of detecting presence of a pancreatic
2 T-type calcium channel in a sample, said method
3 comprising:

4 contacting a sample with the DNA oligomer of claim
5 30, wherein said DNA oligomer hybridizes to any of said
6 pancreatic T-type calcium channel present in said sample,
7 forming a complex therewith; and

8 detecting said complex, thereby detecting presence
9 of a pancreatic T-type calcium channel in said sample.

1 32. The method of claim 31 wherein said DNA
2 oligomer is labeled with a detectable marker.

1 33. An isolated pancreatic T-type calcium channel
2 protein.

1 34. The pancreatic T-type calcium channel protein
2 of claim 33 wherein said pancreatic T-type calcium
3 channel protein is encoded by a nucleotide sequence as
4 shown in SEQ ID NO:1.

1 35. The pancreatic T-type calcium channel protein
2 of claim 33 wherein said pancreatic T-type calcium

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3 channel protein is encoded by an amino acid sequence as
4 shown in SEQ ID NO:2.

1 36. An isolated pancreatic T-type calcium channel
2 protein encoded by a first amino acid sequence having at-
3 least 90% amino acid identity to a second amino acid
4 sequence, said second amino acid sequence as shown in SEQ
5 ID NO:2.

1 37. An antibody or fragment thereof specific for
2 the pancreatic T-type calcium channel protein of claim
3 36.

1 38. The antibody of claim 37 wherein said antibody
2 comprises a monoclonal antibody.

1 39. The antibody of claim 37 wherein said antibody
2 comprises a polyclonal antibody.

1 40. A composition comprising the pancreatic T-type
2 calcium channel protein of claim 36 and a compatible
3 carrier.

1 41. A method of detecting presence of a pancreatic
2 T-type calcium channel protein in a sample, said method
3 comprising:

4 contacting a sample with the antibody or fragment
5 thereof of claim 37, wherein said antibody or fragment
6 thereof binds to any of said pancreatic T-type calcium
7 channel protein present in said sample, forming a complex
8 therewith; and

9 detecting said complex, thereby detecting presence
10 of a pancreatic T-type calcium channel protein in said
11 sample.

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1 42. The method of claim 41 wherein said antibody or
2 fragment thereof is labeled with a detectable marker.

1 43. A method of modifying insulin secretion by —
2 pancreatic beta cells, the method comprising modifying
3 levels of functional T type calcium channels in the
4 pancreatic beta cells.

1 44. The method of claim 43 wherein modifying levels
2 of functional T type calcium channels comprises modifying
3 T type calcium channel gene expression in the pancreatic
4 beta cells.

1 45. The method of claim 44 wherein modifying T type
2 calcium channel gene expression comprises exposing the
3 pancreatic beta cells to a compound which modifies T type
4 calcium channel gene expression.

1 46. The method of claim 45 wherein the compound is
2 an antisense oligonucleotide targeted to the T type
3 calcium channel gene.

1 47. The method of claim 43 wherein modifying levels
2 of functional T type calcium channel comprises exposing
3 the pancreatic beta cells to an inhibitor of the
4 functional T type calcium channel.

1 48. The method of claim 43 wherein modifying levels
2 of functional T type calcium channel comprises exposing
3 the pancreatic beta cells to a compound which interferes
4 with membrane T type calcium channel formation.

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1 49. The method of claim 43 wherein the pancreatic
2 beta cells are present in a subject having type II
3 diabetes.

1 50. A method of treating type II diabetes in a
2 subject, the method comprising administering to the
3 subject an amount of a compound effective to modify
4 levels of functional T type calcium channel in the
5 pancreatic beta cells of the subject.

1 51. The method of claim 50 wherein the compound
2 modifies levels of functional T type calcium channel by
3 modifying T type calcium channel gene expression.

1 52. The method of claim 51 wherein modifying T type
2 calcium channel gene expression comprises exposing the
3 pancreatic beta cells to a compound which modifies T type
4 calcium channel gene expression.

1 53. The method of claim 52 wherein the compound is
2 an antisense oligonucleotide targeted to the T type
3 calcium channel gene.

1 54. The method of claim 50 wherein the compound is
2 an inhibitor of the functional T type calcium channel.

1 55. The method of claim 50 wherein the compound
2 interferes with membrane T type calcium channel
3 formation.

1 56. A method of modifying basal calcium levels in
2 cells, the method comprising modifying levels of
3 functional T type calcium channels in the cells.

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1 57. A method of modifying the action potential of L
2 type calcium channels in cells, the method comprising
3 modifying levels of functional T type calcium channels in
4 the cells.

1 58. A method of modifying pancreatic beta cell
2 death, the method comprising modifying levels of
3 functional T type calcium channels in the pancreatic beta
4 cells.

1 59. A method of modifying pancreatic beta cell
2 proliferation, the method comprising modifying levels of
3 functional T type calcium channels in the pancreatic beta
4 cells.

1 60. A method of modifying calcium influx through L
2 type calcium channels in cells, the method comprising
3 modifying levels of functional T type calcium channels in
4 the cells.

Fig. 1A

Fig. 1B

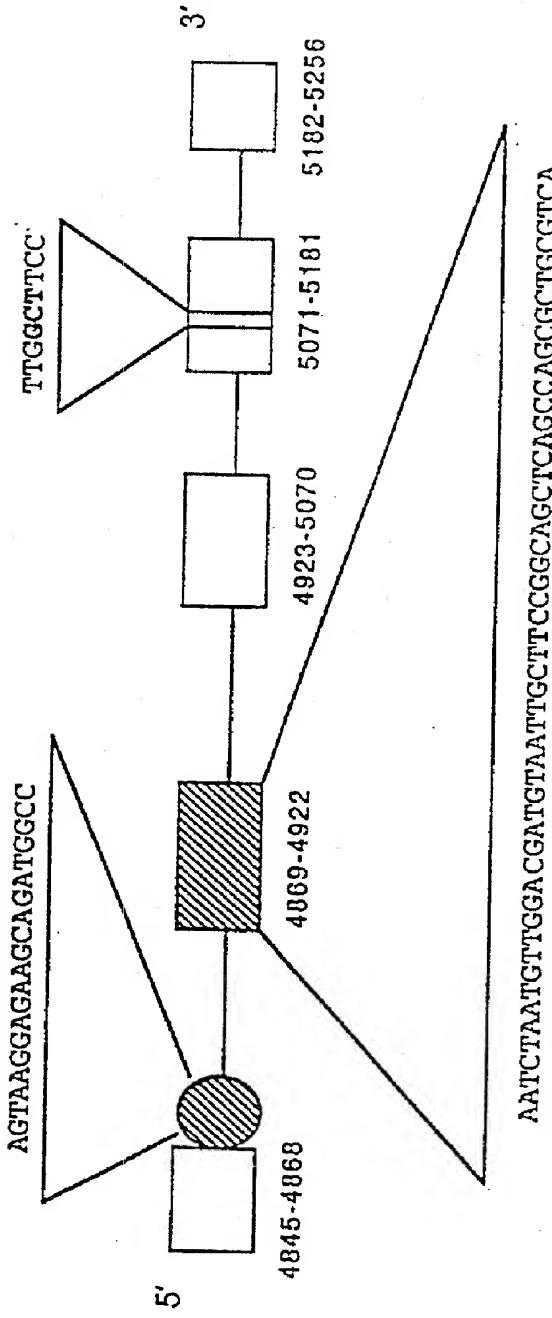


Fig. 2A

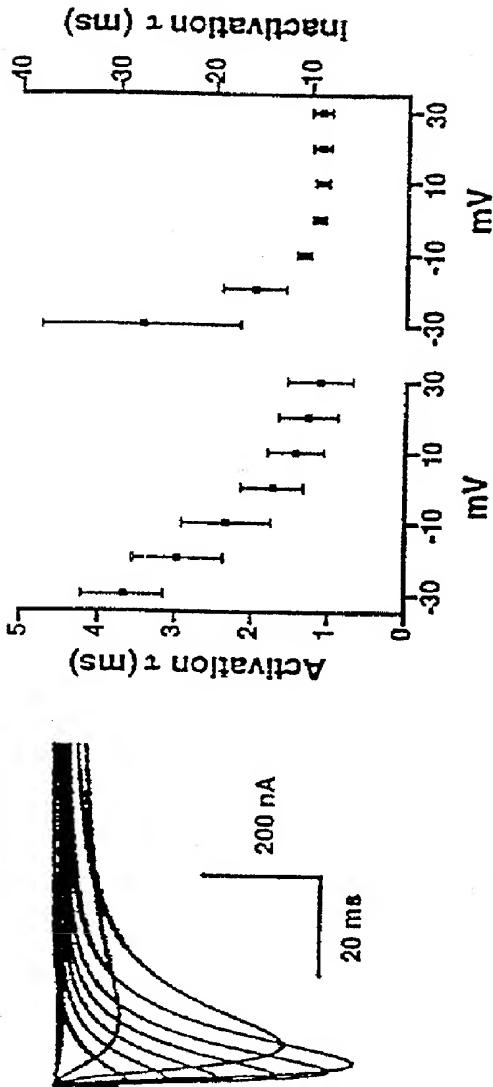


Fig. 2B

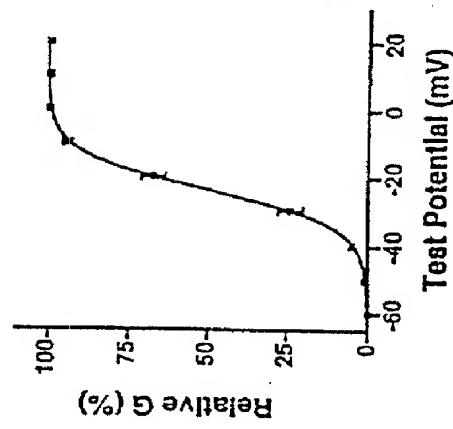


Fig. 2C

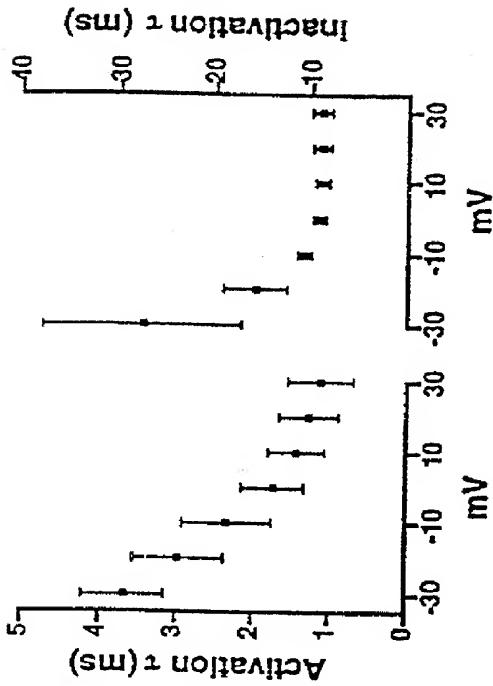
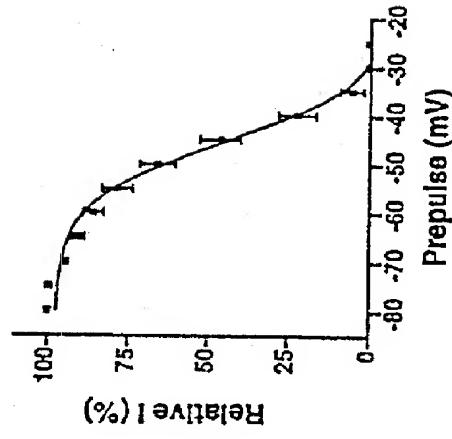
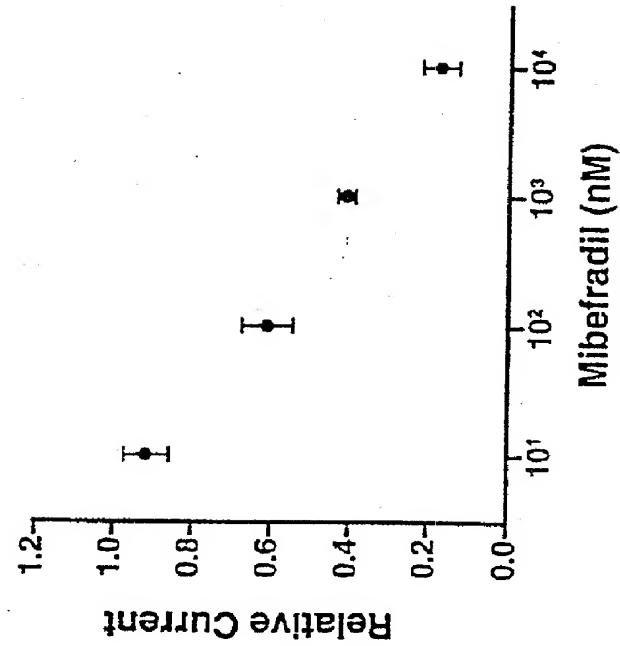
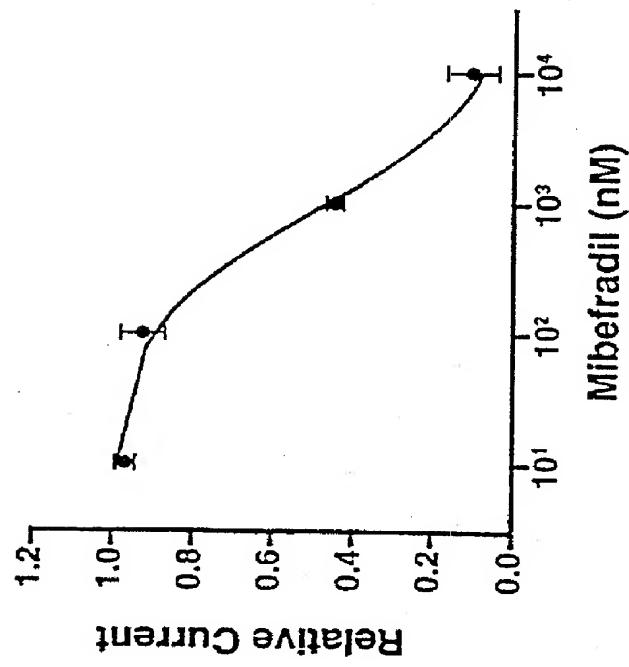
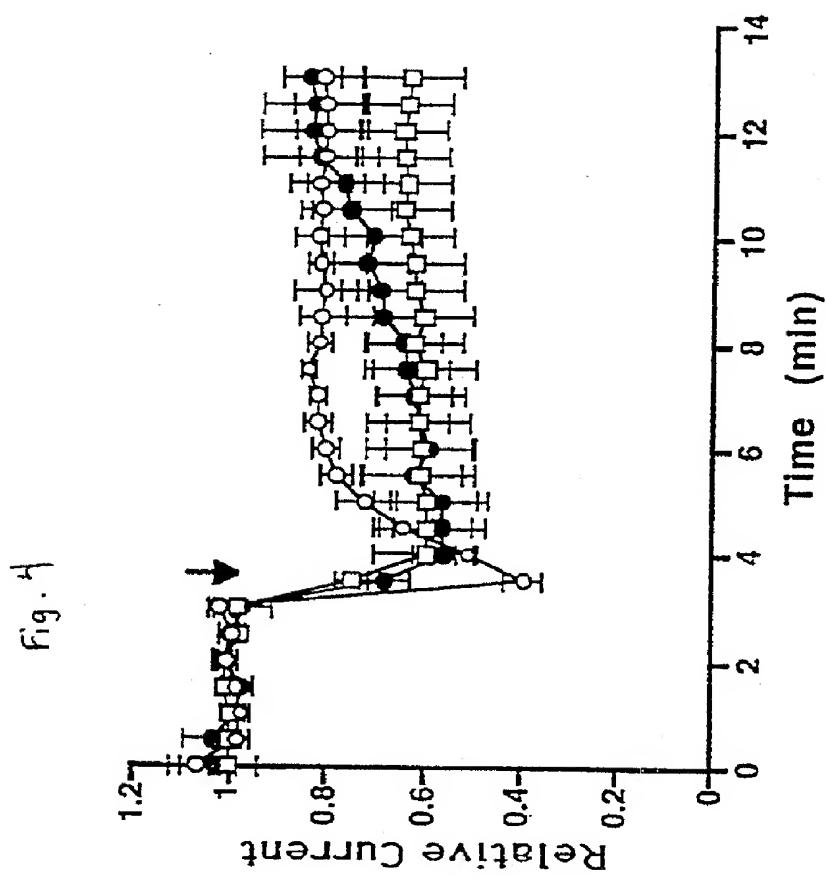
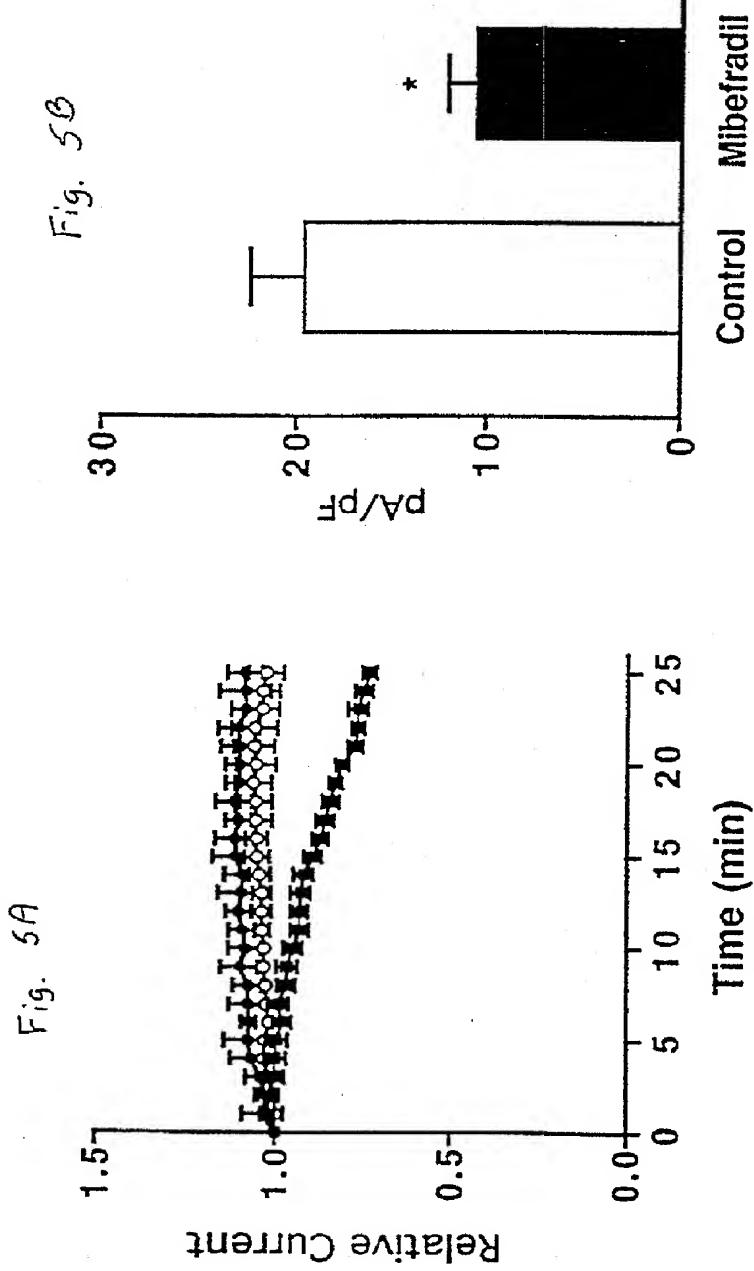


Fig. 3A
Fig. 3B







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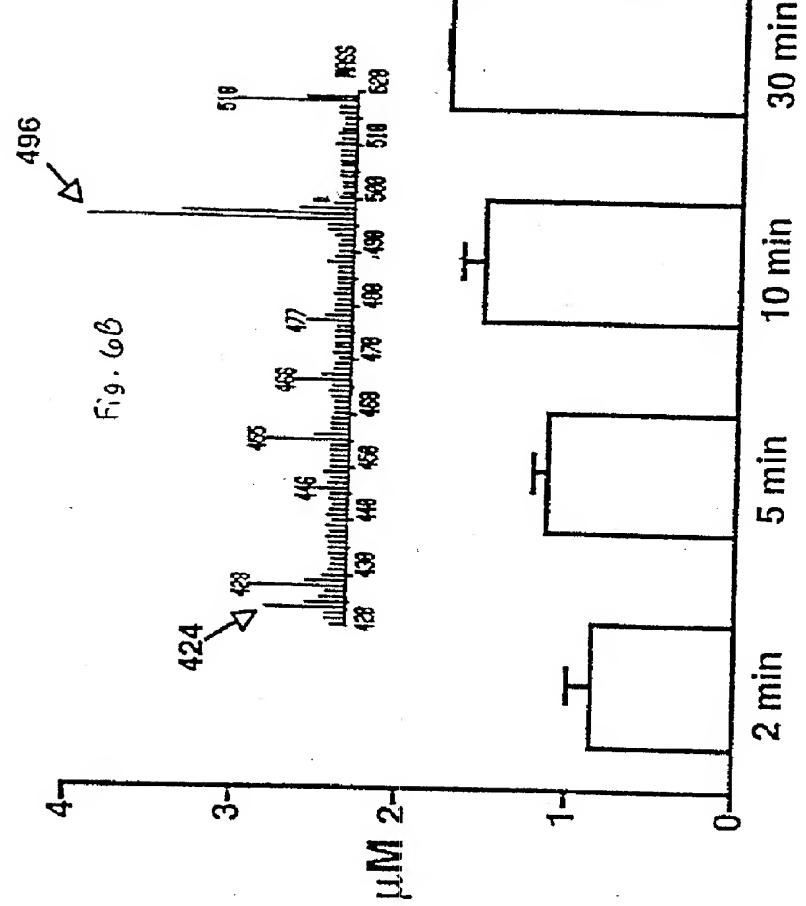


Fig. 6A

Fig. 7B

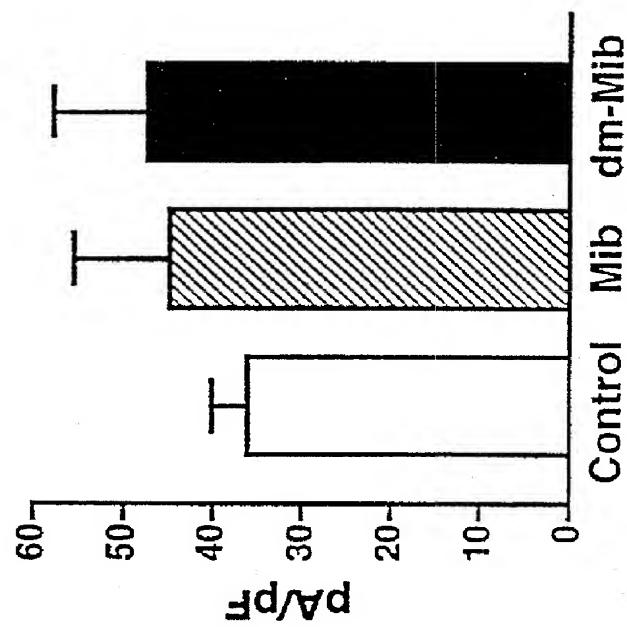
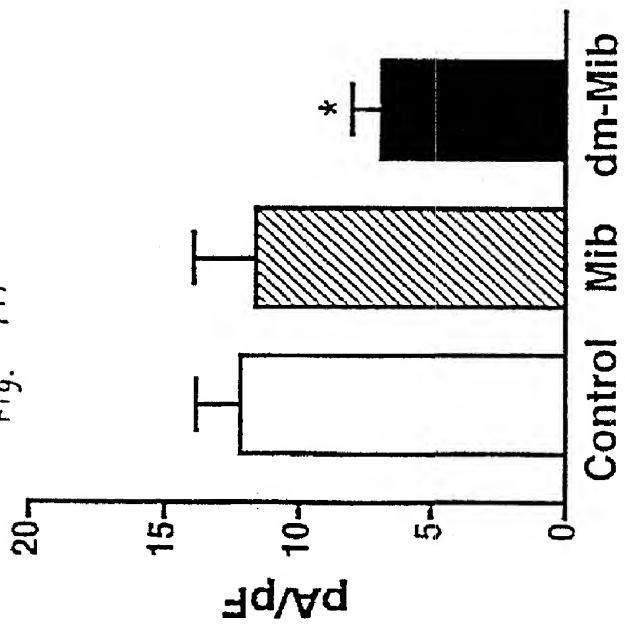


Fig. 7A



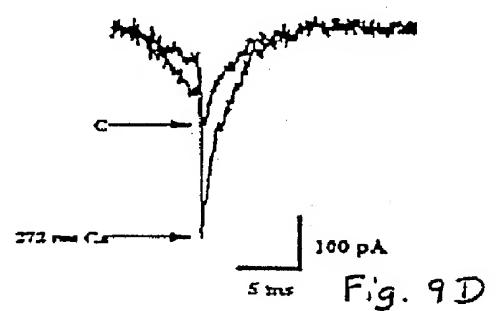
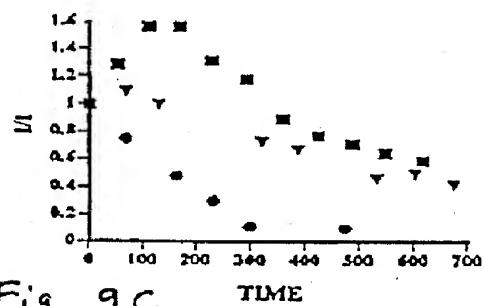
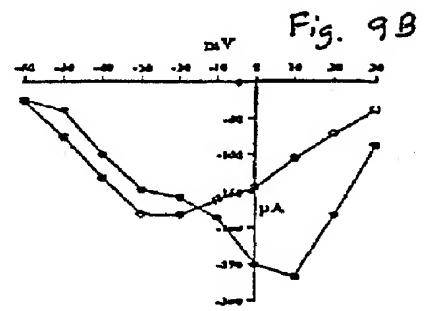
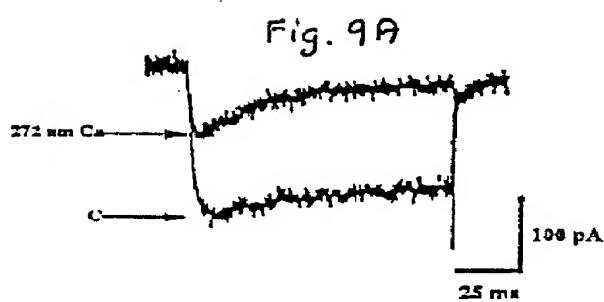
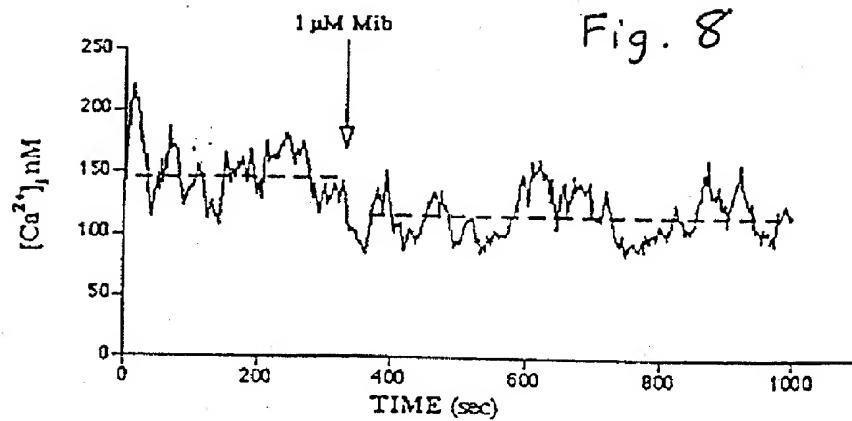


Fig. 10

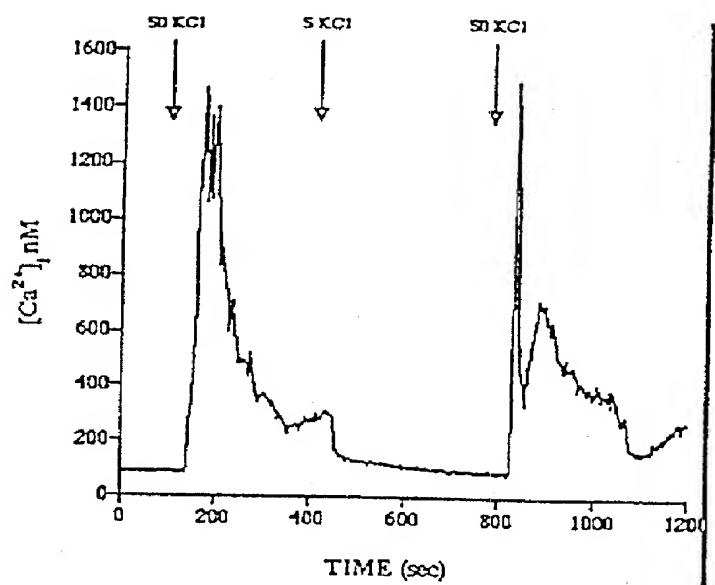


Fig. 11

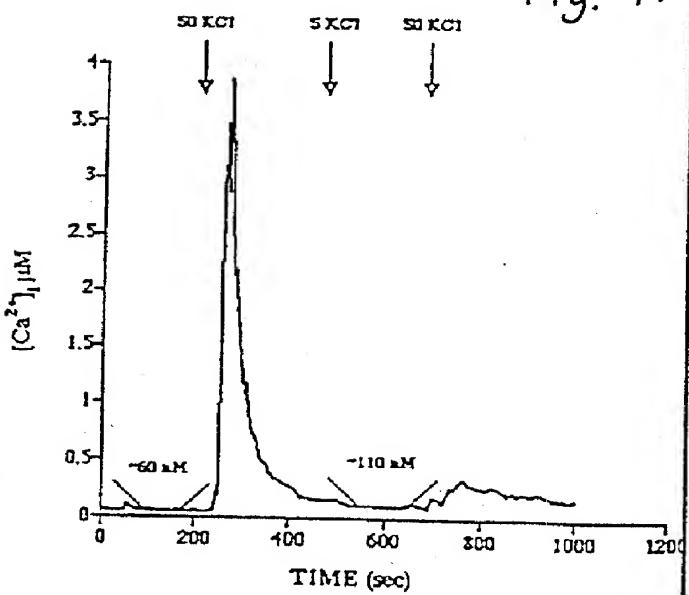


Fig. 12

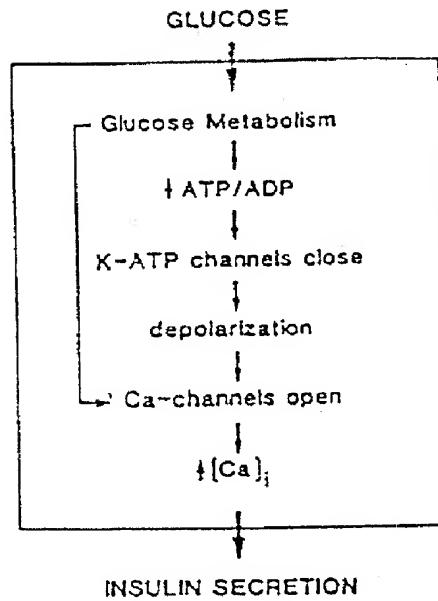
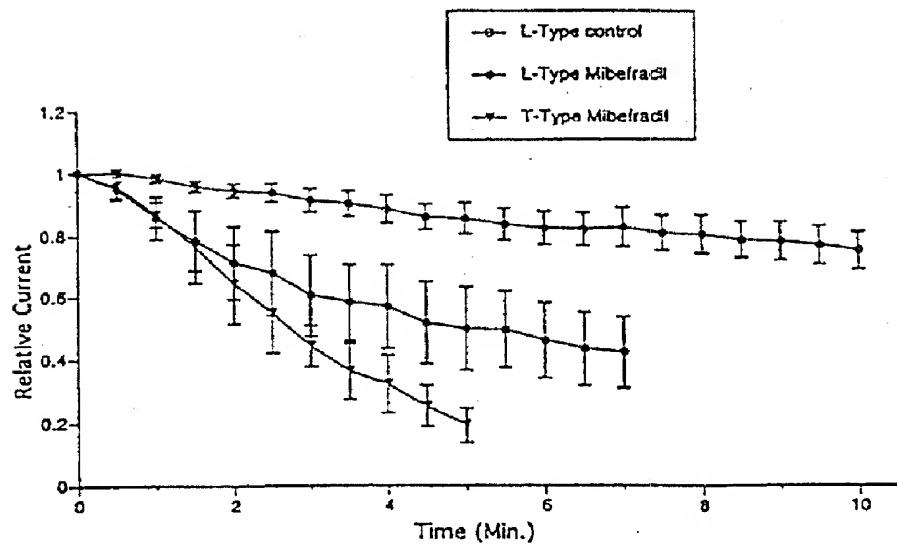


Fig. 13



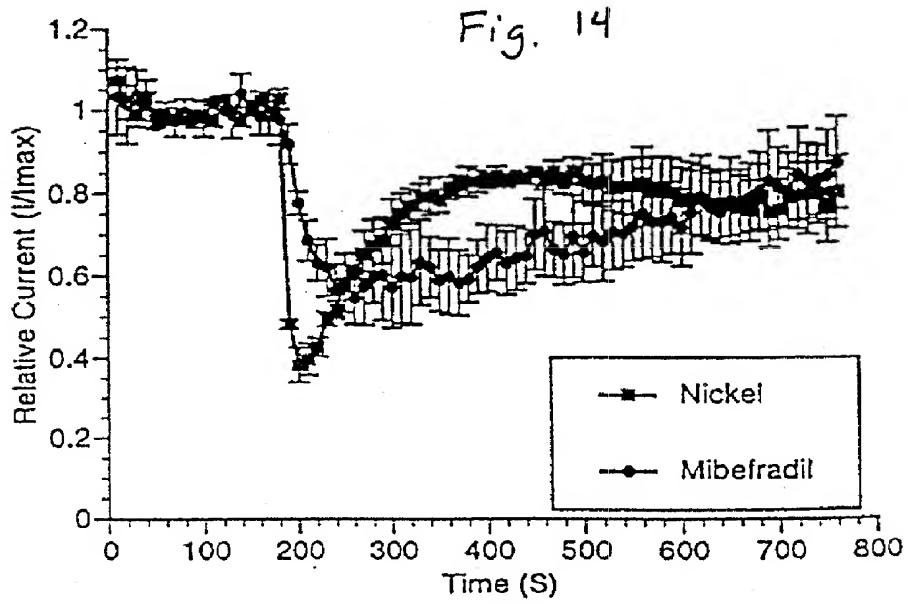


Fig. 15

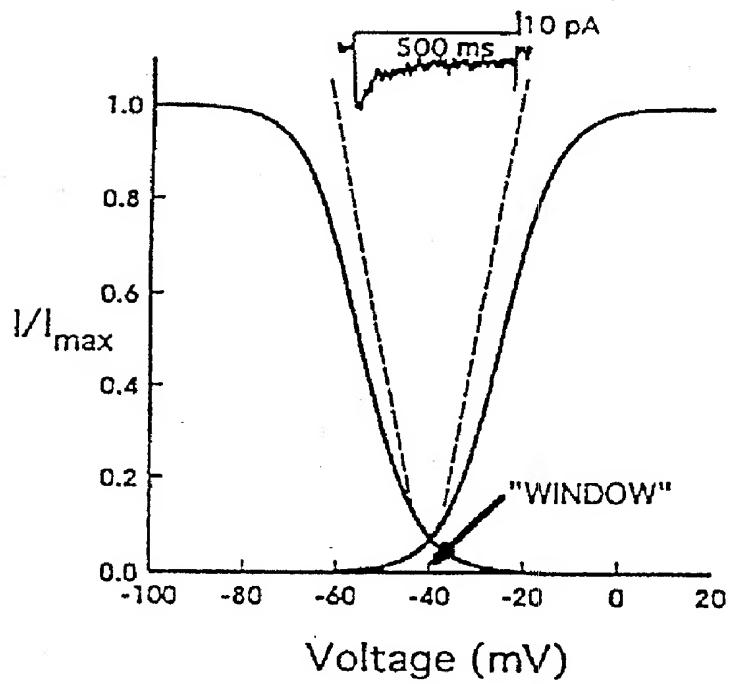


Fig. 16

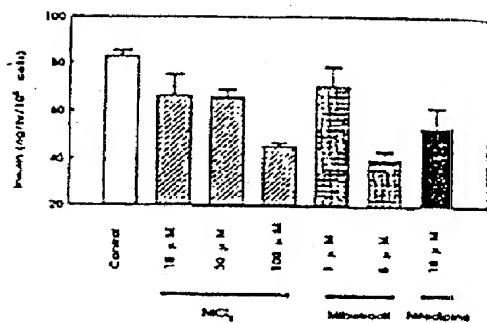


Fig. 17

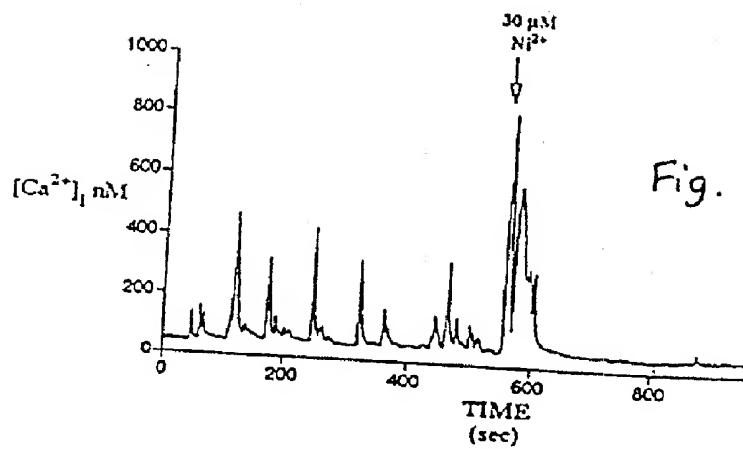
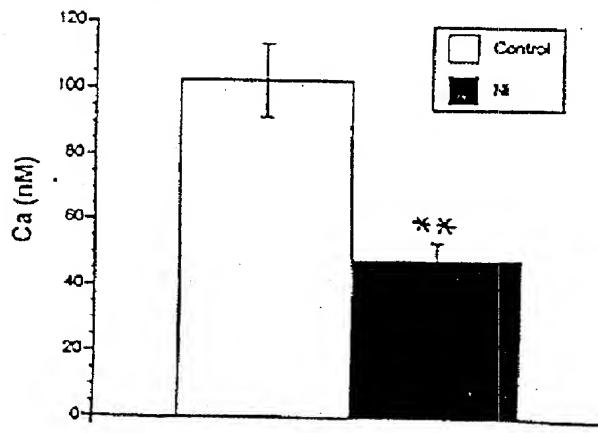


Fig. 18



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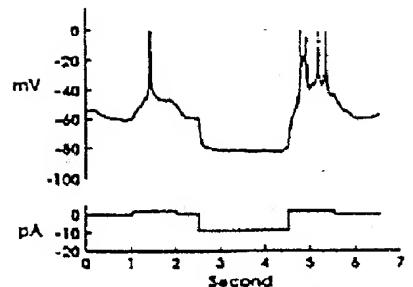


Fig. 19A

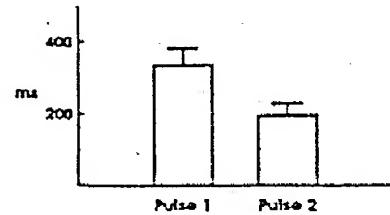


Fig. 19B

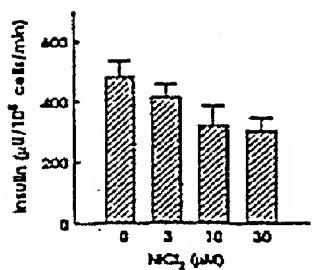


Fig. 20A

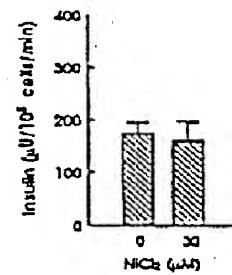
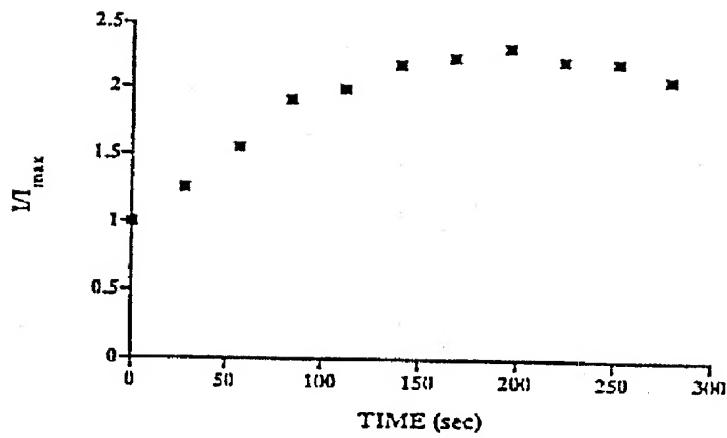


Fig. 20B

Fig. 21



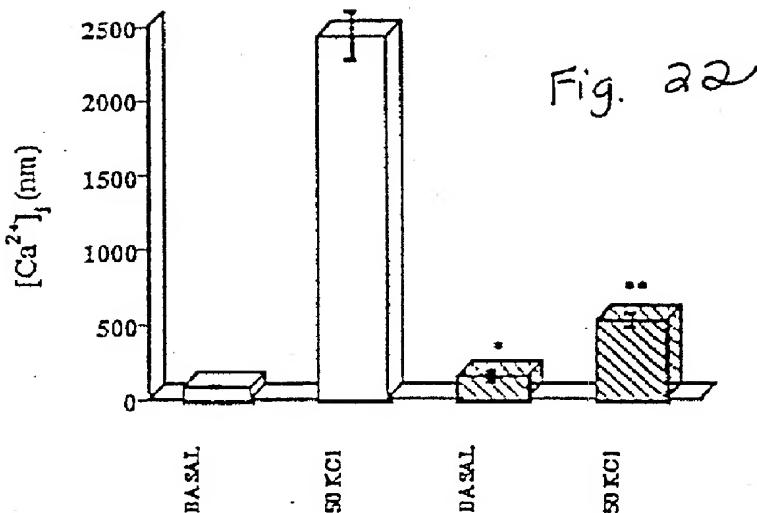
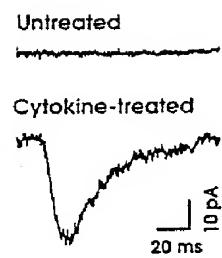
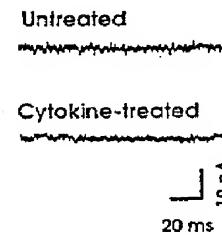
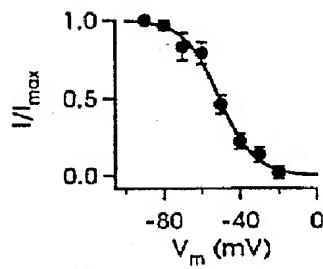
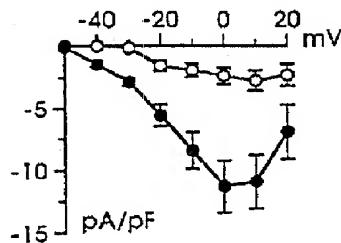
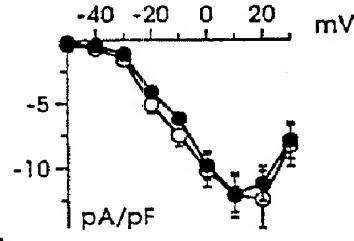
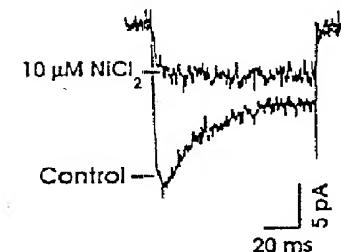
Fig.
23A.Fig.
23C.Fig.
23 E.Fig.
23B.Fig.
23D.Fig.
23 F.

Fig. 24A.

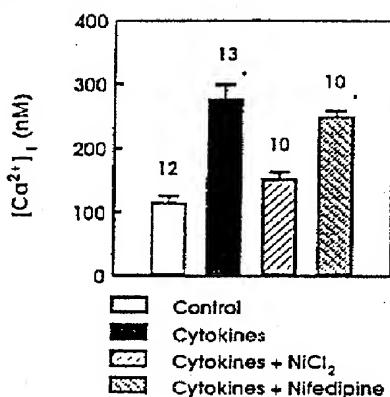


Fig. 24B.

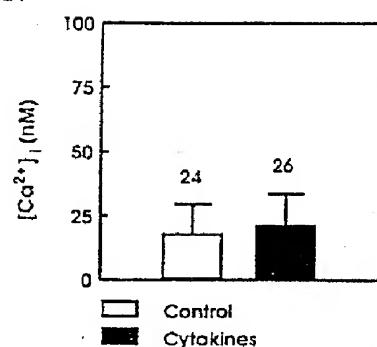


Fig. 25A.

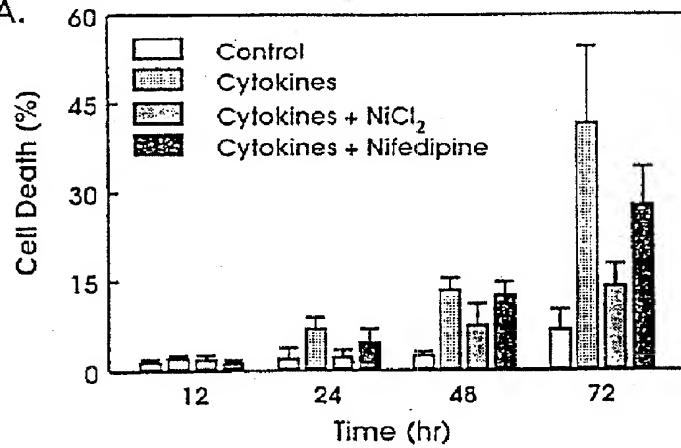


Fig. 25B.

